ALLEN'S
COMMERCIAL ORGANIC ANALYSIS

FOURTH EDITION REWRITTEN AND REVISED

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IN many respects this edition of Allen is a new work. The field of Commercial Organic Analysis has been so enlarged and specialised during the last few years that it has been found necessary to rewrite many parts and add much new matter. Obsolete methods are omitted; what little of the old text remains has been carefully revised and many new illustrations added.

To accomplish the object in view, namely, the furnishing of a modern work of the greatest practical value to the analyst, it was deemed advisable to secure the services of an English and an American editor and to organise a corps of writers particularly versed in the subjects discussed.

The general arrangement of the volumes remains as before, only such changes have been made as will bring the text into line with the latest scientific classification. Great care has been exercised by the editors and contributors in the choice of methods and only those of the highest degree of accuracy and rapidity selected. Effort has been made to secure uniformity in weights and measures, nomenclature and abbreviations. References are to original sources, not to translations or abstracts.

The work is issued in eight volumes, numbered consecutively, as follows:—

DETAIL ARRANGEMENT OF EDITORS, CONTRIBUTORS, SUBJECTS AND VOLUMES

VOLUME I.

EDITED BY HENRY LEFFMANN AND W. A. DAVIS.

Introduction, By W. A. DAVIS; Alcohols, By G. C. JONES; Malt and Malt Liquors, By JULIAN L. BAKER; Wines and Potable Spirits, By G. C. JONES; Yeast, By EMIL SCHLICHTING; Neutral Alcoholic Derivatives, By HENRY LEFFMANN; Sugars, Starch and its Isomers, By E. FRANKLAND ARMSTRONG; Paper and Paper-making Materials, By R. W. SINDALL; Acid Derivatives of Alcohol, By HENRY LEFFMANN; Appendix; Index.

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ALLEN'S
COMMERCIAL ORGANIC ANALYSIS

VOLUME VII

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ALLEN'S COMMERCIAL

ORGANIC ANALYSIS

A TREATISE ON

THE PROPERTIES, MODES OF ASSAYING, AND PROXIMATE
ANALYTICAL EXAMINATION OF THE VARIOUS
ORGANIC CHEMICALS AND PRODUCTS
EMPLOYED IN THE ARTS, MANU-
FACTURES, MEDICINE, Etc.

WITH CONCISE METHODS FOR

THE DETECTION AND ESTIMATION OF THEIR IMPURITIES,
ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

VOLUME VII

Vegetable Alkaloids, Glucosides, Non-Glucosidal Bitter Principles, Animal Bases,
Animal Acids, Lactic Acid, Cyanogen and Its Derivatives

BY THE EDITORS AND THE FOLLOWING CONTRIBUTORS

E. FRANKLAND ARMSTRONG   G. C. JONES   A. E. TAYLOR
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PREFACE.

The last edition of this volume (Vol. III, part iii) was issued in 1896, and, as in the case of Vol. 6, very extensive revision has been necessary in preparing the new edition. In order to interfere as little as possible with the original plan of the work the old arrangement has been retained so far as this has been found practicable. In retaining the old classification the editors have specially considered those chemists who, having become habituated to the order in former editions, look to find information in the new work in much the same position as in the old one.

Prof. W. T. Gies, who had undertaken to revise the section on Animal Bases, was compelled, owing to pressure of other duties, to relinquish this revision, which now appears from the pen of Prof. A. E. Taylor, of the University of Pennsylvania.

The editors are indebted to Dr. H. A. D. Jowett and to Mr. F. H. Carr for perusal and correction of certain of the sections in this volume.
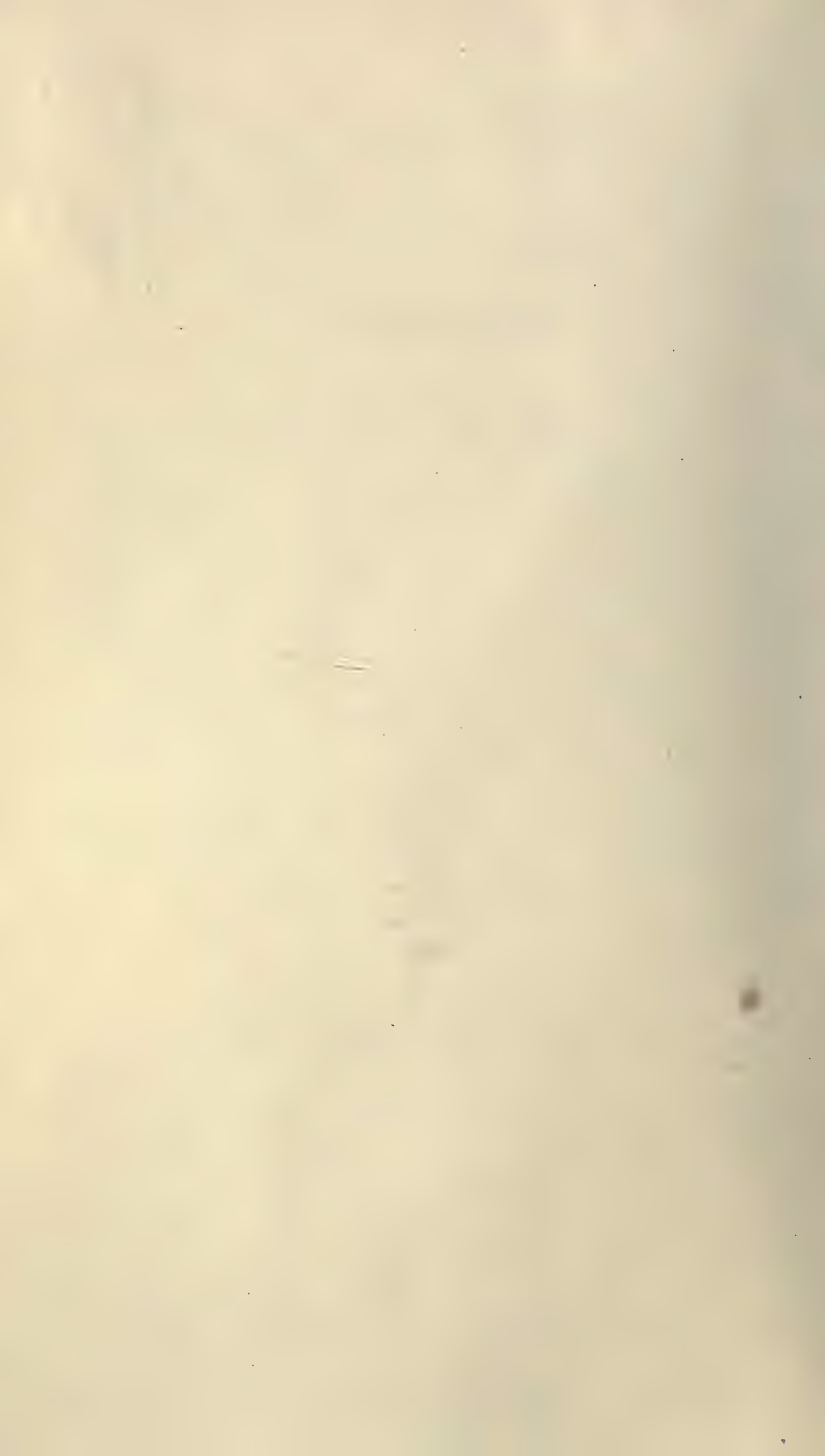
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VEGETABLE ALKALOIDS.

By G. BARGER, M. A., D. Sc.

The more important of the vegetable alkaloids have already been considered (Vol. 6). There remain a number of vegetable bases which are of interest or importance from their employment in medicine (*e.g.*, emetine, physostigmine, pilocarpine); their marked poisonous character (*e.g.*, gelseminine, colchicine); or their occurrence in condiments (*e.g.*, piperine). These will be considered approximately in alphabetical order. Many other alkaloids exist of which little is known, or which are not of sufficient interest or practical importance to require description. In a separate section will be found a description of the more important non-basic bitter principles of vegetable origin.

Alkaloid of Papaya.

Carpaine, $C_{14}H_{25}O_2N$, is contained in the leaves of the Papaya or Papaw tree (*Carica Papaya*),[1] from which it was first isolated by M. Greshoff (*Ber.*, 1890, **23**, 3537; *Pharm. J.*, 1891, [iii], **21**, 560).[2]

According to J. J. L. van Rijn (*Arch. Pharm.*, 1893, **231**, 184), carpaine forms colourless, anhydrous lustrous prisms, melting at 120 to 121° (not at 115°, as stated by Greshoff), and sublimes partially at a somewhat higher temperature. It is practically insoluble in water and in solutions of alkali hydroxides and carbonates. It dissolves readily in absolute alcohol (1:9) and in amyl alcohol, but is very sparingly soluble in dilute spirit. It is soluble in chloroform and carbon disulphide in all proportions, and readily in benzene, but only sparingly (1:100) in petroleum ether. It is but sparingly soluble in ether when once crystallised.

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[1] The same plant yields the trypsin-like ferment called *Papain* or *Papayotin*, which has a digestive action on proteins.

[2] Carpaine is best extracted by digesting the finely-powdered dried leaves of the plant with hydrochloric acid, and subsequently extracting with alcohol. The extract is evaporated to a syrup, treated with acidified water, filtered from the residue of chlorophyll, etc., and shaken with ether to remove resin. The aqueous layer is then made alkaline with sodium hydroxide, and the carpaine shaken out with ether. The yield ranges from 0.07% in adult leaves to 0.3% in young leaves.

Carpaine is dextrorotatory, $[\alpha]_D$ being $+21.55°$ and unaffected by concentration.

Carpaine has an extremely bitter taste, perceptible in dilutions of 1 in 100,000. Carpaine has a depressing action on the heart; it has been recommended as a substitute for digitalis, but has never been employed to any extent. It acts as a heart poison both on frogs and birds.

Carpaine is not removed from acidified solutions by ether or chloroform. Its solutions are alkaline to litmus and cochineal, but have no action on phenolphthalein.

Carpaine yields crystallisable salts. B,HCl forms shining needles, readily soluble in water. B_2,H_2PtCl_6 is yellow and crystalline. The alkaloid is precipitated from very dilute solutions by Mayer's reagent, phospho-molybdic acid, tannin, picric acid, and potassium thiocyanate. B,HAuCl$_4$ crystallises in lemon-yellow needles, m. p. 205°. Bromine-water and iodised potassium iodide give precipitates even with an aqueous solution of carpaine.

With strong mineral acids, either alone or with oxidising agents, carpaine gives no colour indications; except that a mixture of sulphuric acid and potassium dichromate is turned green.

Carpaine appears to be a secondary base, since its compound with ethyl iodide is decomposed by potassium hydroxide forming ethyl-carpaine, $C_{14}H_{24}(C_2H_5)O_2N$, which crystallises from dilute alcohol in colourless silky needles, m. p. 91°. Carpaine is not acted on by either benzoyl chloride or acetyl chloride. With nitrous acid it forms a nitroso-derivative, $C_{14}H_{24}(ON)O_2N$, which crystallises in colourless prisms, m. p. 144° and soluble in alcohol.

By the oxidation of carpaine with sulphuric acid and potassium permanganate, ammonia and an acid free from nitrogen were obtained by van Rijn.

The constitution of carpaine has recently been investigated by Barger (*Trans.*, 1910, **97,** 466) who found that the alkaloid distils without decomposition under a pressure of less than 1 mm. at a temperature of 215 to 235°.

By boiling with concentrated hydrochloric acid, or more readily, by heating with 10% hydrochloric acid or sulphuric acid to 130 to 140° carpaine is quantitatively hydrolysed to *carpamic acid*, $C_{14}H_{27}O_3N$, which forms long needles melting at 224° and subliming unchanged when heated under a pressure of less than 1 mm. Like carpaine it is dextrorotatory, $[\alpha]_D$ in aqueous solution being $+7.0°$.

Unlike carpaine, carpamic acid is quite devoid of bitter taste; it is readily soluble in cold water, but only very sparingly so in alcohol; it is insoluble in acetone, ether, and most organic solvents. Carpamic acid closely resembles certain amino-acids like leucine, and has both acid and basic properties. Thus when carpaine is hydrolysed with hydrochloric acid and the solution evaporated, the *hydrochloride* of carpamic acid, $C_{14}H_{27}O_3N,HCl$, remains behind, and on recrystallisation from acetone forms needles m. p. 161°.

The presence of a carboxyl group in carpamic acid is proved by the readiness with which the substance is esterified by means of alcohol and hydrogen chloride, and when carpaine is heated directly with alcoholic hydrogen chloride there is formed the *hydrochloride* of *ethyl carpamate*, $C_{13}H_{26}ON.COOC_2H_5,HCl$, which forms needles, m. p. 171 to 172°.

On oxidation of carpaine in acetone solution by potassium permanganate, Barger obtained a small quantity of a *dicarboxylic acid*, $C_8H_{14}O_4$, which is obtained in much larger quantity by heating carpaine with 20% nitric acid to 130°, and has been identified as a mixture of the 2 stereo-isomeric forms of α-δ-dimethyladipic acid. It is therefore extremely probable that a dimethyl *cyclo* hexane ring exists in carpaine. The lactone grouping, C.O.CO, must also be present, and these and other facts have led Barger to suggest that carpaine must be constituted in some such way as the following:

$$CH_3 \quad H$$
$$\diagdown\diagup$$
$$C \qquad O$$
$$\diagup\diagdown \qquad$$
$$CH_2 \quad CH \quad CO$$
$$| \qquad | \qquad |$$
$$CH_2 \quad CH \quad C_5H_{10}$$
$$\diagdown\diagup \quad \diagdown\diagup$$
$$C \qquad NH$$
$$\diagup\diagdown$$
$$CH_3 \quad H$$
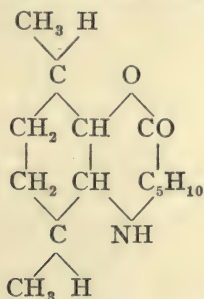
By the action of chlorine on a cold aqueous solution of carpaine hydrochloride, the whole of the alkaloid is precipitated as amorphous chloro-derivative, which is very unstable, but can be crystallised from methyl alcohol, and then forms leaflets, m. p. 77°. It has the composition $C_{14}H_{23}O_3NCl_2$ and is devoid of basic properties; it readily loses chlorine.

Alkaloids of Colchicum.

The meadow saffron, *Colchicum autumnale*, contains a poisonous alkaloid called colchicine. The decomposition-product colchiceïne (page 7) probably occurs naturally in many cases.

Colchicine, $(CH_3O)_3.C_{15}H_9(NH.C_2H_3O).CO_2.CH_3$, is present in all parts of the colchicum plant, but chiefly in the seeds and bulb.

The partial synthesis of colchicine has been effected by heating colchiceïne, methyl iodide, and sodium methoxide together at 100°. Methyl-colchicine is formed at the same time.[1] Colchicine is a pale yellow, amorphous substance, which darkens on exposure to light, and melts with decomposition at 143 to 147°. It dissolves slowly but abundantly in water, forming an intensely bitter, lævorotatory solution. It is readily soluble in alcohol, and in chloroform, and also dissolves in amyl alcohol and hot benzene. It is insoluble in absolute ether and in petroleum spirit.

The basic characters of colchicine are very feebly marked. It is neutral to litmus, and most of its salts are decomposed by water. Hence it is extracted by suitable immiscible solvents, both from acidified and from alkaline solutions.

In presence of a dilute mineral acid or alkali, solutions of colchicine gradually become intensely yellow. Concentrated acids yield a yellow resinous precipitate. Concentrated nitric acid (sp. gr. 1.42) colours colchicine violet-blue, the tint changing to yellow, and ultimately to green. If the violet solution be diluted with water it turns yellow, and changes to a fine orange or red on adding excess of sodium hydroxide. With very minute quantities of colchicine the colouration with nitric acid is rose-red. The test is extremely delicate. Sulphuric acid dissolves colchicine with intense yellow colour, and on adding a drop of nitric acid a dark brown spot is formed, passing gradually through violet and brown to yellow. In an aqueous solution of colchicine, chlorine and bromine water occasion a yellow precipitate which dissolves in ammonia, giving an orange colour.

Colchicine is precipitated very perfectly by tannin and by phosphomolybdic acid. The latter is useful for separating colchicine from solutions containing it. The precipitate gives the foregoing colour-reactions with acids, but if preferred the free base may be obtained by

---

[1] Colchicine forms with chloroform a compound of the formula $C_{22}H_{25}O_6N,2CHCl_3$, which crystallises in needles, which gradually lose chloroform and are readily and completely decomposed into their constituents by boiling with water. Methyl-colchicine forms no similar compound, upon which fact Zeisel founded a separation of the two bases.

agitating the precipitate with ammonia and chloroform, and evaporating the chloroform solution to dryness.

If an aqueous solution of colchicine be treated with powdered manganese dioxide and dilute sulphuric acid, and the liquid filtered after some hours, a filtrate is obtained which acquires a full blue colour on adding excess of ammonia.

An aqueous solution of colchicine gives a brown or yellow precipitate with iodised potassium iodide, and the alkaloid may be conveniently isolated by that reagent. On treating the precipitate with sodium thiosulphate the alkaloid dissolves, and may be extracted from the alkaline solution by chloroform.

Colchicine is precipitated by bismuth potassio-iodide, but not by Mayer's reagent nor by mercuric chloride, unless a mineral acid be added, when the former reagent produces a copious lemon-yellow precipitate. Cadmium potassio-iodide reacts similarly.

Colchicine solutions give no precipitate with platinic chloride, but yield with auric chloride a yellow amorphous precipitate containing $B,HAuCl_4$, which rapidly becomes crystalline, and is readily soluble in alcohol.

An alcoholic solution of colchicine gives a garnet-red colouration with ferric chloride. An aqueous solution gives no immediate reaction, but on warming a green colouration is produced, probably owing to the formation of colchiceïne. In presence of much hydrochloric acid the colouration produced by ferric chloride on heating varies from green to greenish-black. If the liquid be shaken with chloroform after cooling, the latter becomes coloured brownish, garnet-red, or dark and opaque.

An aqueous solution of phenol gives a strong milky turbidity with colchicine, and afterward a yellow resin is precipitated. Acids prevent the reaction.

Picric acid produces no precipitate in a neutral solution of colchicine. On adding dilute sulphuric acid a resinous precipitate is formed, which attaches itself to the sides of the vessel.

E. Barillot (*Bull. Soc. Chim.*, 1894, [iii], **11,** 514) has described the following mode of operating, which he claims to afford a very reliable test for colchicine, and a means of distinguishing it from morphine and codeine. A minute quantity of the free alkaloid, in the form of an ether or chloroform residue, is mixed very intimately with 0.25 grm. of oxalic acid and 1 c.c. of strong sulphuric acid. The mixture is

sealed up in a small glass tube, which is kept at 120° for one hour. Subsequently the tube is opened, the colour of the mixture observed, and excess of alcoholic sodium hydroxide added. Operating in this way, the following results are stated to be obtained:

Alkaloid	Colouration in the cold	Colouration after heating	Observations
Colchicine..	Golden yellow..	Dark reddish-brown, not modified by addition of water.	On treating the aqueous solution with an alkali and reacidifying, a yellow precipitate is obtained, which is soluble in chloroform;[1] on evaporating this solution a yellow residue is obtained, which becomes violet-red on treatment with nitric acid (sp. gr. 1.42) and raspberry-red with strong sulphuric acid.
Morphine...	Light blue......	Reddish-brown..	On addition of a large volume of water the colour sometimes changes into blue. Treating this solution with potassium hydroxide and alcohol, then acidifying it, and shaking it with chloroform, the latter assumes a blue colour. Ether takes a purplish-red colour. On evaporating these two solvents morphine-blue remains behind.
Codeine....	Bright blue.....	Greenish-blue...	Like morphine.

Barillot states that none of the ptomaïnes shows any similar reaction. With 1 mgrm. of colchicine the above test can be repeated ten times.

Colchicine is very unstable, being decomposed on heating with alkalies or dilute acids with elimination of methyl alcohol and formation of colchiceïne. The latter substance is formed so readily that some of the reactions commonly attributed to colchicine itself are probably due to its decomposition-product. By further treatment, as by increasing the strength and proportion of the acid, the colchiceïne itself undergoes further change, with elimination of an acetyl and one or three methyl groups, producing compounds which, together with the higher terms of the series, are formulated below.

For a knowledge of these compounds we are chiefly indebted to Zeisel (*Monatsh.*, 1883, **4,** 162; 1886, **7,** 557; 1888, **9,** 1, 865).

Colchic acid. Colchicinic acid, $(OH)_3 : C_{15}H_9 \begin{cases} NH_2 \\ CO.OH \end{cases}$

Dimethylcolchic acid, $(O.CH_3)_2 (OH) : C_{15}H_9 \begin{cases} NH_2 \\ CO.OH \end{cases}$

[1] If the colouring matter is not entirely taken up by the chloroform, but floats about in flakes, it may be collected on a very small filter, and the latter dried, cut into strips, and immersed in the acid.

Trimethylcolchic acid,	$(O.CH_3)_3 : C_{15}H_9$ $\begin{cases} NH_2 \\ CO.OH \end{cases}$	
Acetyl-trimethylcolchic acid. Colchiceïne,	$(O.CH_3)_3 : C_{15}H_9$ $\begin{cases} NH.C_2H_3O \\ CO.OH \end{cases}$	
Methyl acetyl-trimethylcolchate. Colchicine,	$(O.CH_3)_3 : C_{15}H_9$ $\begin{cases} NH.C_2H_3O \\ CO.O(CH_3) \end{cases}$	
Methyl-colchicine,	$(O.CH_3)_3 : C_{15}H_9$ $\begin{cases} N(CH_3).C_2H_3O \\ CO.O(CH_3) \end{cases}$	

Colchiceïne, or acetyl-trimethylcolchic acid, $C_{21}H_{23}O_6N$, is a substance possessing both acid and feebly basic characters. It is best obtained by warming colchicine with water containing 2% of sulphuric acid or 1% of hydrochloric acid. On cooling, the colchiceïne separates in shining white needles, containing $1/2$ H_2O. It becomes anhydrous at 140 to 150°, softens at 161° and melts at 172°. It is readily soluble both in acids and alkalies, with yellow colour. Colchiceïne is soluble in hot water and very soluble in alcohol and chloroform; but insoluble in ether and benzene. The solutions of colchiceïne are lævorotatory. They are coloured yellow by alkalies and mineral acids, and concentrated nitric and sulphuric acids behave as with colchicine. The solution in hydrochloric acid reacts for the most part like colchicine. If sufficiently concentrated, auric chloride throws down an orange-coloured aurichloride, $B,HAuCl_4$, crystallising in needles. Bromine-water, phenol-water, and phosphomolybdic acid give slight precipitates with aqueous solutions of colchiceïne, but most other alkaloidal reagents give negative results. Precipitates are produced by lead and copper acetates. Ferric chloride gives a fine green colouration with a dilute acidified solution of colchiceïne.

When colchiceïne (or colchicine) is heated in the water-bath with 3 or 4 parts of hydrochloric acid of 1.15 sp. gr., acetic acid is evolved, and a mixture of the hydrochlorides of colchic acid and its di- and tri-methylated derivatives is obtained. When a trial portion of the solution ceases to become turbid when mixed with water, the product is diluted with a little water and shaken twice with chloroform, which extracts the hydrochloride of trimethylcolchic acid and any unchanged colchiceïne only. The chloroform solution is evaporated, the residue taken up with water, and the separation of unchanged colchiceïne promoted by adding a crystal of that substance. The filtered solution is again shaken with chloroform, which now takes up colchiceïne only,

and the aqueous liquid leaves the hydrochloride of trimethylcolchic acid on evaporation. On treating the last aqueous solution with an amount of caustic potash sufficient to react with the hydrochloric acid only, free trimethylcolchic acid separates on cooling.

Trimethylcolchic Acid, $C_{19}H_{21}O_5N + 2H_2O$, forms minute yellow prisms, m. p. 159°. It closely simulates colchiceïne in its behavior. Ferric chloride produces a garnet-red colouration with green dichroïsm. On further addition of the reagent or of hydrochloric acid, the colour becomes green, and on shaking with chloroform the same reaction is obtained as with colchicine. Trimethylcolchic acid forms a *hydrochloride*, crystallising in glistening, yellowish-white plates, moderately soluble in cold water to form a yellow solution. $B_2, H_2 PtCl_6 + 2H_2O$ forms small yellow needles, and $B,HAuCl_4$ a brown precipitate. The solutions also give precipitates with chlorine-, bromine- and iodine-water, picric acid, and cadmium iodide.

Dimethylcolchic acid, $C_{18}H_{19}O_5N + 4\frac{1}{2}H_2O$, produced together with the last substance, forms minute yellow prisms, m. p. 141°. $B,HCl + H_2O$ forms sparingly soluble, white, microscopic needles. Its solution is precipitated by most of the alkaloidal reagents, except platinic chloride and tannin. It dissolves in concentrated sulphuric acid with orange colour, and on addition of a minute quantity of a nitrate gives the same colour-reactions as colchicine. With ferric chloride it behaves like trimethylcolchic acid.

Colchic Acid, or Colchicinic Acid, $C_{16}H_{15}O_5N$, is the final product of the action of strong acid on colchicine, and is obtained by heating that body or colchiceïne with 4 parts of fuming hydrochloric acid at 140°. The resultant hydrochloride is very soluble in water, and yields the free "acid" on fractional neutralisation with potassium hydroxide. Colchic acid forms brown flakes, the solution of which is precipitated by most of the alkaloidal reagents. With ferric chloride the solution in hydrochloric acid gives an intense brownish-red colour, which is not taken up by chloroform. On adding a trace of potassium nitrate to the solution in concentrated sulphuric acid, a dull red colour is produced, which changes to a splendid red on the addition of excess of ammonia.

Assay of Colchicum. Estimation of Colchicine.

The parts of the meadow saffron which contain colchicine in largest proportion are the seeds and the corm. The British Pharmacopœia

directs the employment of the seeds for the preparation of the *tincture* (which is made with proof-spirit), and the corm for that of the *extract* or *wine* of colchicum.

In addition to colchicine, which is the only important medicinal constituent, colchicum *corms* or *tubers* contain starch (about 10%), gum, sugar, tannin, and fatty, resinous and colouring matters. The *seeds* contain much sugar, a small proportion of an acid resembling gallic acid, and from 6 to 8% of fixed oil. Colchiceïne is probably present in many cases.

J. Hertel (*Pharm. Zeit. f. Russland*, 1881, **20**, 245; *Year-Book Pharm.*, 1882, 78) recommends, for the extraction of colchicine, that the uncrushed seeds should be exhausted with 85% alcohol.[1] Powdered seeds are stated to give an extract loaded with foreign matter and to yield less alkaloid. The solution is shaken with calcined magnesia, filtered after a few hours, and the filtrate distilled in a vacuum. The extract is diluted with about 10 volumes of water, filtered from oily matter, and repeatedly shaken with chloroform. The chloroform is separated, distilled off, and the syrupy residue poured on glass plates and heated to 80 to 100° for an hour, or until it is no longer soft and waxy when hot (indicative of the presence of chloroform, which is retained very obstinately). The brown brittle mass thus obtained is dissolved in 20 parts of water, and the filtered solution evaporated to dryness. The yield of alkaloid obtained by Hertel from colchicum seeds, working in this manner, was from 0.38 to 0.41%

For the preparation of colchicine, Hübler (*Arch. Pharm.*, 1865, **171**, 193) exhausts the seeds with boiling alcohol, and dilutes the resultant solution with 20 volumes of water to precipitate fatty matters. The filtered liquid is treated with basic lead acetate, and the excess of lead removed by sodium phosphate. The colchicine is precipitated from the filtrate by tannic acid, which is said to form a compound of definite composition. The moist precipitate is mixed with oxide of lead and the mass treated with boiling alcohol, which on evaporation leaves the colchicine as a yellow bitter powder.

For the *assay* of colchicum seeds, Farr and Wright recommend that 20 grm. of the powdered material should be exhausted by cold percola-

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[1] Other operators prefer proof-spirit to stronger alcohol, and insist on the necessity of using a hot solvent. R. A. Cripps (*Pharm. J.*, 1891, [iii], **22**, 364) has shown that the amount of alkaloids extracted when uncrushed seeds are used is little more than one-fourth cf the quantity obtained when the powdered drug is used. Nevertheless Blau (*Zeit. Oesterr. Apoth-Verein*, 1903, **41**, 1067, 1091, 1119) states that the colchicine is only present in the seed coat and that it is unnecessary to crush the seeds.

tion with spirit of 50%, 25 c.c. of water added to the extract, and the
alcohol evaporated at a moderate temperature. The residual liquid
is diluted to 20 c.c., and when quite cold slightly acidified with sul-
phuric acid (5 c.c. $N/10$ H_2SO_4). It is then twice agitated with
petroleum-ether to remove oil, and colouring matter, and the separated
aqueous liquid made slightly ammoniacal and agitated twice with
chloroform, the separated chloroform evaporated, and the residual
alkaloid dried at 100° and weighed. The proportion of alkaloid found
in the seeds by Farr and Wright (*Pharm. J.*, 1891, [iii], **21**, 957) by this
process ranged from 0.46 to 0.95%. This variation in alkaloidal
strength extends to the official tincture of colchicum, which causes an
uncertainty as to its potency highly objectionable in the case of so
active a preparation. Carr and Reynolds (*Pharm. J.*, 1908, [iv], **26**,
542) by a process not described found the colchicine content of the
seeds to vary from 0.12 to 0.57%.

The United States Pharmacopœia (1905) requires a colchicine con-
tent of 0.45% in the seeds, and 0.35% in the corms. The *assay*
method for the corms is as follows: 10 grm. in No. 60 powder is macer-
ated, with occasional shaking for 12 hours (or is shaken continuously
for 4 hours) with 100 c.c. of a mixture of 77 c.c. ether, 25 c.c. chloro-
form, 8 c.c. alcohol and 3 c.c. ammonia water. After filtering 50
c.c. (= 5 grm. of the corm) is collected and evaporated. The residue
is dissolved in 10 c.c. ether, and after adding 5 c.c. water and stirring,
the ether is evaporated on the water-bath. After cooling the solution
is filtered into a small separator. As much as possible of the precipi-
tate is retained in the dish or beaker, and is redissolved in 5 c.c. of
ether and treated as before with water. Filtrate and washings are
shaken in the separator successively with 15, 10, 10 and 10 c.c. of
chloroform, and the 4 extracts evaporated together. The residue is
dissolved in alcohol, the latter is evaporated. The above process is
now gone through again; the residue is therefore dissolved in 5 c.c.
of ether, 5 c.c. of water are added, and after stirring the ether is evap-
orated; the water is again filtered into a separator and the substance
in the filter is washed with 5 c.c. of water. The filtrate is extracted
successively with 15, 10, 10 and 10 c.c. of chloroform, the latter is
evaporated, the residue is dissolved in alcohol, evaporated and dried
at 100°. The weight of the residue multiplied by 20 gives the per-
centage of colchicine in the corm.

A simpler method for the estimation of colchicine in the corm has

lately been published by A. B. Lyons (*Pharm. J.*, 1909, [iv], **28**, 270). 25 grm. of the corm, in a moderately fine powder, are treated with 15 c.c. of solution of lead subacetate (United States Pharmacopœia) and 80 c.c. of warm distilled water. After macerating with occasional stirring for 6 hours at 50°, the mixture is transferred to a small percolator tube and percolated with warm water until 250 c.c. of liquid have been collected. To the percolate enough powdered sodium phosphate is then added to precipitate all the lead, which is filtered off. Of the filtrate 100 c.c. (= 10 grm. of the drug) are shaken successively with 25, 20 and 15, c.c. of chloroform. The chloroform is evaporated, alcohol is repeatedly added to the residue and again evaporated to remove last traces of chloroform and the residue is dried at 100° and weighed as alkaloid.

The main difficulty in the assay of colchicine is due to the feebly basic nature of the alkaloid, which makes a sharp separation by means of acids impossible.

Toxicology of Colchicum.—Colchicine has valuable medicinal properties, and in excessive doses is a powerful poison. The free alkaloid is not official, but is employed in the forms of extract or wine prepared from the corms, or of a tincture of colchicum seeds. Colchicum is the active ingredient of certain proprietary remedies for the treatment of gout, but is regarded with grave mistrust by many competent authorities, owing to the variable effects produced by it. These are probably in great measure due to the uncertain alkaloidal strength of the official tincture and other preparations. Colchicine augments the excretion of uric acid, and reduces the quantity contained in the blood; but its accumulation in the system and marked toxicity render great caution necessary in its administration. The elimination of colchicine from the system occurs chiefly by the kidneys, but is very slow; and hence small doses, not poisonous in themselves (0.00016 grm. per kilogrm. of body-weight), may cause death within 5 days. Colchicine causes congestion in the articular extremities and the marrow of the bones.

According to Mairet and Combemale (*Compt. Rend.*, 1887, **104**, 439, 515), colchicine acts, according to the dose, either as a diuretic or as a purgative, and in excessive quantities is an irritant poison, affecting more especially the digestive canal and the kidneys, though capable of irritating any of the organs. Its therapeutic effects are the same whether administered subcutaneously or by the stomach, but in the

former case the action is more rapid and the dose required is smaller. In proportion to their weight, men are three times more susceptible to colchicine than are dogs or cats, the total dose required to produce diuresis in man being 0.002 to 0.003 grm., and for a purgative effect 0.005 grm. The fatal hypodermic dose of colchicine for dogs and cats is 0.000571 grm., and when taken internally 0.00125 grm., per kilogrm. of the weight of the animal.

Poisonous doses of colchicum occasion all the symptoms of violent gastro-intestinal irritation, including griping, vomiting, diarrhœa, prostration, and painful spasms of the limbs and trunk (but without tenderness of the abdomen), followed by resolution and collapse, without delirium or coma. On *postmortem* examination, the most notable symptoms are lividity of the skin in depending parts, engorgement of the veins, dark pitchy blood in the lungs, brain and trunk, sometimes a dark-coloured injection of the gastrointestinal mucous membrane, and more or less shedding of the intestinal epithelium. The kidneys are pale.

In searching for colchicum, it must be remembered that the active principle colchicine readily suffers decomposition by heating with alkalies or dilute mineral acids, and that it is extracted from both alkaline and acidified aqueous liquids by agitation with chloroform, but not by petroleum-spirit, which latter solvent may consequently be employed to remove fatty and resinous matters. When isolated in a fairly pure state, the chemical reactions of colchicine and its decomposition-product colchiceïne are sufficient for the recognition of the poison.

For the identification of the alkaloid when thus isolated, Obolonski (*Zeit. anal. Chem.*, **29**, 493) prefers the violet colouration with nitric acid; the reaction with Erdmann's reagent (Vol. 6, page 200), which gives, in succession, green, dark-blue, violet, and yellow colourations, turning to raspberry-red on adding alkali; and the green colour produced by sulphovanadic acid (Vol. 6, page 449). Obolonski states that colchicine is with difficulty destroyed by putrefaction of animal matter, and that the kidneys, bladder, and urine are best suited for toxicological examination.

A non-poisonous substance giving many of the reactions of colchicine has been found among the normal constituents of beer, being apparently derived from the hops (*Archiv der Pharm.*, 1876, **208**, 411; *Pharm. J.*, 1876, [iii], **7**, 351. See, however, *Zeit. anal. Chem.*, 1877, **16**, 116, 328).

Alkaloid of Laburnum and Furze.

Cytisine, $C_{11}H_{14}ON_2$, is contained in the seeds, bark, and other parts of the laburnum (*Cytisus laburnum*), and several other species of the same genus. It is identical with the alkaloid of furze (*Ulex Europæus*), sometimes called ulexine.

To isolate cytisine, A. Partheil (*Ber.*, 1891, **24,** 634) recommends that laburnum-seeds should be extracted with 60% alcohol acidified with acetic acid, the alcohol distilled off, and the extract dissolved in water. The solution is passed through a wet filter to separate oil and resin, and then precipitated by lead acetate. The filtered liquid is then rendered strongly alkaline by potassium hydroxide, and shaken with chloroform or amyl alcohol. The separated solvent is agitated with dilute hydrochloric acid, and the acid solution evaporated to dryness. On treating the residue with cold absolute alcohol almost all the colouring matter is separated, and by repeatedly crystallising the residual cytisine hydrochloride from water it is obtained in colourless, well-defined crystals. On decomposing the salt with concentrated potassium hydroxide and extracting with chloroform, the free alkaloid is obtained as a pale yellow oil, which quickly solidifies to a crystalline mass, and may be further purified by recrystallisation from absolute alcohol or boiling petroleum-spirit.[1] Lead acetate is, however, unnecessary and the best method is to extract with 94% alcohol, concentrate, dissolve the residue in dilute acid, filter, concentrate, add alkali hydroxide and extract with chloroform. By working in this way on 160 lbs. of seeds, 1.4% of the crystalline base was obtained. (Private communication of Mr. F. H. Carr.)

The constitution of cytisine has not been definitely ascertained, but one of the atoms of nitrogen is in secondary combination, as shown by the behaviour of the base with methyl iodide, acetic anhydride, and nitrous acid. The other nitrogen atom is either in tertiary or quaternary combination; while the oxygen exists neither as hydroxyl nor methoxyl, no acetyl-derivative being obtainable from methyl-cytisine on treatment with acetic anhydride. On distillation of cytisine with soda-lime, a base of the formula $C_9H_{13}N$ is obtained, which is probably a pyridine-derivative.

Cytisine forms large, transparent, odourless, anhydrous prismatic needles or laminæ, m. p. 152 to 153°, and can be sublimed unchanged.

[1] From the ripe seeds of *Cytisus laburnum*, Partheil isolated about 1.5% of cytisine, while the leaves and fruit gave smaller amounts. Choline was also found, but the base, soluble in ether mentioned by Gerrard and Symons could not be obtained.

It is extremely soluble in water, alcohol, and chloroform, but less readily in benzene, amyl alcohol, or acetone; and is nearly insoluble in ether, petroleum-spirit, or carbon disulphide. Benzene dissolves 1.26, and amyl alcohol 0.30%. In aqueous solution, cytisine is strongly alkaline, and exhibits the optical activity $[\alpha]_D = -119.6°$.

Cytisine is a strong base, displacing ammonia from its salts even in the cold. All the ordinary salts are crystallisable and soluble. B,HCl and $B(HCl)_2 + 2\ 1/2H_2O$ have been described. Two platini-chlorides are known; B_2,H_2PtCl_6 forming pale yellow, lustrous, sparingly soluble plates or needles, and $B,H_2PtCl_6 + 2\ 1/2H_2O$ golden yellow, tolerably soluble needles, which decompose when heated without previously melting. $B,HAuCl_4$ crystallises in short, reddish-brown needles, which melt with decomposition at 212 to 213°.

Cytisine gives precipitates with the usual alkaloid reagents. Bromine-water gives an orange-red precipitate in very dilute solutions (1:15,000), as also do phosphomolybdic and phosphotungstic acids (1:30,000). The most characteristic reaction of cytisine and its salts is that with ferric chloride, which produces a red colouration. This is destroyed by hydrogen peroxide, but on then warming the liquid a blue colouration is immediately produced. This reaction is very characteristic, and is said to be produced by 0.00005 grm. of cytisine.

Another characteristic reaction of cytisine is that observed by Magalhaës, which consists in adding thymol to a solution of cytisine in concentrated sulphuric acid, and heating, when a yellow colouration, finally passing into intense red, is produced.

With strong sulphuric acid, cytisine affords no colour-reaction, but on adding potassium dichromate a yellow colour, changing to brown, is produced, or with nitric acid an orange-yellow. It does not reduce phospho-molybdic acid.

Cytisine possesses marked poisonous properties. A case is recorded of a child having been poisoned by milk from a cow which had fed on laburnum, though cytisine has been ascertained to be relatively innocuous to cattle. All parts of the laburnum contain cytisine, and cases are on record of poisoning by the seeds, flowers, bark, and twigs. The prominent symptoms of poisoning are vomiting, purgation, prostration, hallucinations, muscular tremors and a condition of collapse with pallor and cold sweats. The pupil is usually dilated. The clinical picture is very similar to that of nicotine poisoning, and it has recently been shown experimentally by Laidlaw (*Proc. Roy. Soc. Med.*,

Therapeutical and Pharmacological Section, 1911, **5**, 10) that the action is indeed similar to that of nicotine and lobeline in almost all respects. This paper also contains references to the toxicological and pharmacological literature on cytisine.

In toxicological inquiries, cytisine is best isolated by extraction with chloroform from a solution containing excess of alkali hydroxide. The urine and vomit are the most likely to contain the alkaloid. Salts of cytisine are excreted by the kidneys within twenty-four hours, but if laburnum seeds or leaves have been employed, the elimination may take a longer time.

Cytisine is present in the bark and young tops of furze, but in smaller proportion than in the seeds, from which Gerrard isolated 0.19%, while Partheil (*Ber.*, 1891, **24**, 634) obtained as much as 1%.

Gerrard has obtained indications of the presence in furze-seeds of a second alkaloid which is soluble in ether.

Sophorine, an alkaloid contained in *Sophora tomentosa*, is regarded by Greshoff as not improbably identical with cytisine (*Arch. Pharm.*, 1891, **229**, 561).

Alkaloids of Stavesacre.

The seeds of *Delphinium Staphisagria* contain several optically inactive, very poisonous alkaloids, which have last been investigated by Charalampi Kara-Stojanow (*Pharm. Zeit. f. Russland*, 1890, **29**, 628; abstr. *J. Chem. Soc.*, 1891, **60**, 842) and by Ahrens (*Ber.*, 1899, **32**, 1581, 1669).

According to Couerbe the finely powdered seeds are extracted with 4 to 5 parts of 90% alcohol containing tartaric acid. After evaporation of the alcohol, an oil is separated off and the solution is extracted with light petroleum; it is then rendered slightly alkaline with sodium hydrogen carbonate and extracted with ether.

Delphinine, $C_{31}H_{49}O_7N$, crystallises from the ethereal solution in rhombic crystals, which decompose at 120° without melting. It has intensely bitter, acrid taste, and does not yield colour reactions.

Delphisine, $C_{31}H_{49}O_7N$, is isomeric with delphinine and also forms rhombic crystals. It is said to give no colour-reactions.

Delphinoïdine, $C_{42}H_{68}O_7N_2(?)$, is amorphous and remains in the delphinine mother-liquors. It is soluble in sulphuric acid with a reddish-brown colour and green fluorescence. Mixed with malic acid,

and treated with sulphuric acid, it yields an orange coloured mass.
The solution in concentrated sulphuric acid, treated with bromine
water, yields a weak fugitive violet colouration, changing to yellow.

Staphisagrine is, according to Kara-Stojanow, a mixture.

Staphisagroïne, $C_{40}H_{46}O_7N_2$, is present in minute quantity
(Ahrens). It is a powder almost insoluble in most solvents and melting
at 275 to 277°.

For cats and dogs the lethal dose of delphinine is 1.5 mg., for delphi-
sine 0.7 mg., for delphinoïdine 5 mg. per kilo of body weight. Delphi-
nine has been used in neuralgia, earache and toothache. The dose is
stated to be 1/2 grain. The seeds of stavesacre are no longer official.
The toxicity of delphinine is primarily due to its effect on respiration;
it has an asphyxiating action resembling that of aconitine.

The Alkaloids of Ergot.

For many years the only alkaloid of ergot isolated in a pure condi-
tion was the *ergotinine* of Tanret (*Compt. Rend.*, 1875, **81,** 896). This
crystalline alkaloid is physiologically inert; more recently Barger and
Carr (*Trans.*, 1907, **91,** 337) were able to crystallise a number of
salts of a second, amorphous alkaloid, named by them *ergotoxine* on
account of its powerful physiological action. They were also able to
establish definitely the formulæ of the two alkaloids, ergotinine being
$C_{35}H_{39}O_5N_5$ and ergotoxine $C_{35}H_{41}O_6N_5$, so that the latter is the
hydrate of the former, in accordance with the view first expressed by
Kraft (*Arch. Pharm.*, 1906, **244,** 336) who succeeded in transforming
the amorphous alkaloid, called by him *hydro-ergotinine*, into crystalline
ergotinine by boiling with methyl alcohol.

For an account of synonyms of ergotinine, and of the impure alka-
loidal preparations of previous investigators, such as ergotine, ecboline,
cornutine, sphacelinic acid and sphacelotoxin, see Barger and Carr
(*loc. cit.*), and Barger and Dale (*Biochem. Journ.*, 1907, **2,** 240); the
latter paper contains an account of the physiological action of the ergot
alkaloids.

Various processes have been suggested for the extraction of the ergot
alkaloids. For the preparation of ergotinine on a small scale, it is
best to take advantage of the fact that it can be extracted from the
powdered drug by ether. On evaporation of the ether there is left
a fatty oil, representing about 33% by weight of the ergot employed,

and in the oil remain dissolved the ergotinine, yellow colouring matters, resin, etc. By mixing the crude oil with light petroleum the alkaloid is precipitated along with the colouring matter. The precipitate represents Jacobj's chrysotoxin and from it the alkaloids may be obtained by repeated extraction with citric, tartaric or acetic acids. The alkaloids may also be obtained by shaking the concentrated ethereal extract of the drug with an aqueous solution of a weak acid, rendering the aqueous layer alkaline with sodium carbonate and shaking again with ether. On evaporation of the ether the ergotinine generally crystallises out, and may readily be obtained quite pure by recrystallisation from absolute alcohol; the ergotoxine remains in the mother liquor. Working as above, it is essential to use a weak organic acid. When a strongly ionized acid, such as hydrochloric, is employed, the hydrochloride, which forms a colloidal solution in distilled water, is precipitated out by the excess of the acid employed; it forms an emulsion between the aqueous and ethereal layers and is thus lost.

The slight solubility of the hydrochloride and particularly of the hydrobromide may, however, be put to account when working on a larger scale, particularly with a view to isolating ergotoxine. The drug is extracted with 96% alcohol and the residue left on evaporation of the alcohol is extracted with light petroleum to remove oily matter; it is then dissolved in ethyl acetate and shaken with citric acid solution. Sodium bromide or hydrobromic acid is added to the citric acid solution, and the precipitated hydrobromides of the alkaloids are collected. A rough separation of ergotinine from ergotoxine is effected by repeated shaking of the solution of the mixed hydrobromides in dilute sodium hydroxide with ether; in this way the ergotinine is removed first, and can be obtained pure by recrystallisation from alcohol. The ergotoxine which contains a carboxyl group and has acidic properties, mostly remains in the sodium hydroxide solution, which is neutralised and again rendered alkaline with sodium carbonate. The ergotoxine can now be extracted by ether; the residue after evaporation of the ether, together with that from the ergotinine mother liquors, is dissolved in 80% alcohol and a slight excess of phosphoric acid in alcohol is added. On standing for some days ergotoxine phosphate crystallises out and can be recrystallised from alcohol. It is best to use 50 c.c. of boiling 90% alcohol for 1 grm. of the phosphate. From absolute alcohol the salt crystallises much less readily.

Ergotinine, $C_{35}H_{39}O_5N_5$, crystallises in long needles the sides of

which are not quite parallel; the ends are symmetrically replaced by a pair of faces and the extinction is straight (Fig. 1).

Placed in a bath previously heated to 210°, ergotinine melts at 230 to 231° (uncorr.).

At 18°, it dissolves in 292 parts of ethyl alcohol, in 1,020 parts of absolute ether, in 91 parts of ethyl acetate and in 26 parts of acetone; further, in 77 parts of boiling benzene, in 52 parts of boiling ethyl alcohol and in 56 parts of boiling methyl alcohol. It is extremely soluble in chloroform and almost insoluble in light petroleum.

Ergotinine is strongly dextrorotatory; the specific rotation of a saturated alcoholic solution at 10° (0.257%) giving $[\alpha]_D + 338°$.

Fig. 1.—Ergotinine.

On boiling the solution of ergotinine in alcohol transformation to the ethyl ester of ergotoxine gradually takes place, accompanied by a fall in the rotation of the solution. To minimise loss of material, the recrystallisation of the alkaloid from alcohol should be carried out rapidly.

The basic properties of ergotinine are feeble and no crystalline salts are known.

Ergotoxine (hydro-ergotinine) is a white amorphous powder, softening at 155° and melting at 162 to 164°. It is more soluble in organic solvents than ergotinine, notably in cold alcohol.

Ergotoxine phosphate, $C_{35}H_{41}O_6N_5,H_3PO_4,H_2O$, is more easily purified than any other known ergotoxine salt. Crystallised from boiling 90% alcohol, it forms slender needles (Fig. 2), which are generally arranged in groups radiating from a centre; when not quite pure sphero-crystals are formed. The salt melts at 186 to 187° with decomposition (the bath being heated to 180° before the introduction of the substance). The salt dissolves in 313 parts of cold and in 14 parts of boiling 90% alcohol. By shaking ergotoxine phosphate with cold distilled water a 1% opalescent solution is readily obtainable, but

this colloidal solution is converted into a jelly by the addition of electrolytes, such as salts or strong acids.

Ergotoxine hydrochloride, $C_{35}H_{41}O_6N_5.HCl$, is best prepared by adding alcoholic hydrogen chloride to an ethereal solution of the base, and crystallising the precipitate by carefully adding dry ether to its solution in cold 90% alcohol. The salt forms long square-ended needles m. p. 205° (Fig. 3).

Fig. 2.—Ergotoxine Phosphate.   Fig. 3.—Ergotoxine Hydrochloride.

The *normal oxalate*, $(C_{35}H_{41}O_6N_5)_2,H_2C_2O_4$, prepared in a similar manner, forms rectangular plates, m. p. 179°.

Ergotoxine contains a carboxyl group and can be esterified (Barger and Ewins, *Trans.*, 1910, **97**, 284). Several crystalline salts of ergotoxine ethyl ester have been prepared by heating ergotinine in alcoholic solution with a slight excess of various acids. The *phosphate*
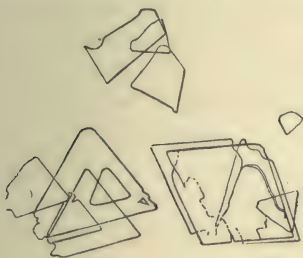
Fig. 4.—Phosphate of Ergotoxine   Fig. 5.—Hydrochloride of Ergotoxine
Ethyl Ester.   Ethyl Ester.
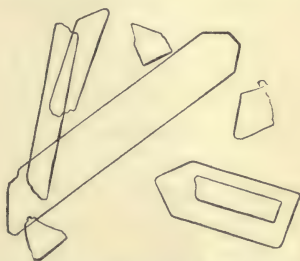
$C_{37}H_{45}O_6N_5,H_3PO_4$, m. p. 187 to 188°, and the *hydrochloride*, $C_{37}H_{45}O_6N_5,HCl$, m. p. 206 to 207°, are shown in figures 4 and 5.

Ergotoxine ester is also formed on heating ergotinine with alcohol, and as the free ester is amorphous and readily soluble in alcohol, this accounts for the loss which is generally experienced in recrystallising ergotinine from that solvent.

Ergotinine is readily converted into ergotoxine by the action of alkalies and acids in aqueous solution; the reverse change is brought about by heating ergotoxine with methyl alcohol or with acetic anhydride.

On destructive distillation, preferably under reduced pressure, both ergot alkaloids yield a small quantity of a crystalline and very volatile sublimate, which is *iso*butyrylformamide, $CH(CH_3)_2.CO.CO.NH_2$ (Barger and Ewins, *loc. cit.*). Neither alkaloid contains a phenolic hydroxyl or a methoxyl group.

Ergotinine and ergotoxine give precipitates with all the usual alkaloidal reagents in very dilute solution (*Trans.*, 1907, **91**, 348). Mayer's reagent is the most sensitive, even producing a faint opalescence in dilutions of 1 part per million. For the quantitative recognition of both ergot alkaloids a characteristic colour-reaction, originally described by Tanret for ergotinine, consists in allowing a solution of 1 mgrm. of the alkaloids in ether or in ethyl acetate to evaporate on the surface of concentrated sulphuric acid; a transitory orange colouration is produced, changing to violet-blue. The reaction depends on the presence of oxides of nitrogen in the sulphuric acid. Keller has improved this test as follows:

A small quantity (0.002 to 0.003 grm.) of the solid alkaloid is dissolved on a watch-glass in a few drops of sulphuric acid and a drop of ferric chloride solution adhering to a glass rod, or a trace of the anhydrous salt is stirred in; the solution acquires an orange-red colour, changing to deep red and at the edge a blue or greenish colour gradually appears. An alternative is to add a trace of anhydrous ferric chloride to a solution of about 1 mgrm. of the alkaloids in 2 to 3 c.c. of glacial acetic acid; on the careful addition of concentrated sulphuric acid, so as to avoid mixing, an intense blue colouration is produced at the boundary of the two layers. According to Keller this test still yields a positive result with the crude alkaloid extracted from 1 grm. of ergot; it has been adopted in the last edition of the Dutch Pharmacopœia.

Tanret (*Compt. Rend.*, 1909, **149**, 222) has recently isolated an interesting base from ergot, called *ergothioneine* on account of its sulphur content. It occurs to the extent of about 0.1% and crystallises from water in leaflets, m. p. 290°, having the composition $C_9H_{15}O_2N_3S,2H_2O$; $[\alpha]_D + 110°$. The substance dissolves in 8.6 parts of water at 20°; it is a feeble base. The *hydrochloride*, $C_9H_{15}O_2N_3S,HCl,2H_2O$,

has $[\alpha]_D = +88.5°$ and melts at 250°.   Solutions of ergothioneine salts give precipitates with mercuric chloride, potassium mercuric iodide and platinic chloride in excess, but not with picric or tannic acids. Ergothioneine does not cause any of the physiological effects characteristic of ergot.

Barger and Ewins (*Trans.*, 1911, **99**, 2336) have recently shown that ergothioneine is β-2-thiolglyoxaline-4 (or 5)-propiobetaine and therefore closely related to histidine.

Estimation of the Ergot Alkaloids.

It will be clear from the above description of the properties of ergotinine and ergotoxine, that it is hardly possible to estimate either alkaloid separately by chemical means.   It is possible, however, to estimate the total alkaloid by some such method as Keller's, and an idea of the ergotoxine content may be obtained by physiological experiment.

Keller (*Schweiz. Wochenschr. f. Chem. u. Pharm.*, 1894, **32**, 121, and dissertation) extracts 25 grm. of the finely powdered ergot with light petroleum, until a few drops of the percolate leave no residue on evaporation.   The powder is then dried thoroughly at a gentle heat, is placed in tared stoppered bottle of 250 c.c. capacity, and is extracted with 100 grm. of ether. After 10 minutes milk of magnesia is added, prepared by shaking 1 grm. of magnesium oxide with 20 c.c. of water. After the addition of magnesia the mixture is at once thoroughly shaken for some time, until the ergot powder cakes together and the ethereal solution is clear.   The shaking is frequently repeated in the course of half an hour and then 80 grm. of ether are poured off (or if this is impossible a smaller quantity which is corrected for by calculation, 4 grm. of ether being equivalent to 1 grm. of ergot).   If the solution is not quite clear, which happens in rare instances, it is left standing for some hours, and then decanted.

The ethereal solution which has thus been obtained is extracted successively with 25, 15 and 10 c.c. of 0.5% hydrochloric acid.[1]   After a fourth shaking, with 10 c.c. of acid, the latter should not give an opalescence with Mayer's reagent.   If it does, it is added to the other quantities of acid.   The acid extract may be somewhat turbid, but this is no disadvantage; it is shaken with an equal volume of ether

[1] Phosphoric or citric acids are doubtless to be preferred.

after an excess of ammonia has been added and this is repeated with two further volumes of ether. The mixed ethereal extracts are then filtered and evaporated in a weighed flask on the water-bath. The residue is twice treated with ether which is boiled off, and the residue is then dried to constant weight. In six different commercial specimens of ergot Keller found by this method 0.095 to 0.245% of alkaloid (which he regarded erroneously as being wholly ergotinine).

Keller's method has been somewhat modified by Fromme (Caesar und Loretz, *Geschäftsbericht*, 1908, 116) and in the trade reports of this firm a considerable number of alkaloid estimations in ergot have been published, *e.g., Geschäftsbericht*, Sept., 1905 (see *Pharm. Centralhalle*, 1905, **46,** 859).

In 1904 the extreme limits were 0.025 to 0.414%, the better kinds varying between 0.28 and 0.37%. In 1905 these limits were 0.013 to 0.38 and 0.28 to 0.35 respectively. Beckurts and Grothe (*Pharm. Zeitung*, 1896, **41,** 210) found less alkaloid in German ergot than in the other kinds examined, and found also relatively less in the larger sclerotia than in the smaller ones. The smaller alkaloidal content of larger sclerotia has been confirmed by Mjoen and it would appear that the generally assumed superiority of "bold" ergot is very doubtful.

In 1899 a committee of the German *Apotheker Verein*, appointed to consider the new edition of the Pharmacopœia, found 0.0976 to 0.327% of alkaloid and suggested a legal minimum of 0.15%. If we exclude a few quite abnormally low results we may state, in round numbers, that the alkaloidal content of ergot varies from 0.1 to 0.4% and is generally between 0.2 and 0.3%.

Physiological Action of the Ergot Alkaloids.

Pure ergotinine, as such, is almost inactive, but it may undergo change in the organism to ergotoxine, at least when given by the mouth. Ergotoxine is one of the chief active principles of ergot, producing on intravenous injection in mammals a somewhat prolonged rise of blood pressure and also contraction of the uterus, on which its therapeutic application depends. The prolonged administration of doses too small to be immediately fatal, produces gangrene (generally studied experimentally in the cock's comb). Ergotoxine is the active principle responsible for ergotism, an epidemic disease due to the eating of bread from ergotised rye. For an account of the physiological properties of

ergotoxine and a physiological method of estimating it, see Dale (*J. Physiol.*, 1906, **34**, 163; Barger and Dale, *Biochem. J.*, 1907, **2**, 240). The other chief active principles of ergot are described in the section dealing with ptomaïnes (see *p*-hydroxyphenylethylamine and *β*-iminazolylethylamine, this Volume, pages 346 and 349.

Detection and Estimation of Ergot in Flour.

Although this question is hardly of great importance in England, where so little rye bread is eaten, reference may nevertheless be made to some of the methods which have been suggested by continental writers for the detection of ergot in flour.

The amounts of ergot to be looked for are of the order of a few per cent. 3% has been suggested as the highest permissible amount in flour (J. Moeller, *Zeit. Nahr. Genussm.*, 1895, 157); this would correspond to something like 9% of ergot in corn (comp. Musset, *Pharm. Central-Halle*, 1899, **40**, 353), an amount which is not very much below that observed in the corn during various epidemics. We may therefore conclude with Hanausch, that Moeller's limit is too high.

It is best first to submit a suspected flour to microscopical examination. Musset takes a mixture of about 10 vols. of chloroform and 1 vol. of absolute alcohol and by addition of one or other of the constituents adjusts its sp. gr. to 1.435 at the temperature of the experiment. 5 grm. of the flour is shaken with 60 c.c. of this mixture in a tall cylinder. On standing the ergot remains floating and can be poured off with the upper portions of the fluid; after diluting the chloroform with alcohol, in another vessel, the ergot sinks to the bottom and can be examined in xylene under a high power.

Another, half quantitative process, also suggested by Musset, depends on the estimation of the alkaloidal content. 200 grm. of dry flour is left for 2 hours under a bell jar with a little strong ammonia, and is then extracted with ether, first by shaking in a bottle with 200 c.c. of ether and then by percolating with 450 c.c. The crude alkaloid is obtained by shaking the ether with acid, rendering the extract alkaline with ammonia and shaking with ether, in the way described above (Keller's method for the determination of alkaloids in ergot). Half the residue from the ether is then tested by Keller's reaction (see above); the other half is extracted by acid and made up to 50 c.c. If the addition of 3 drops of Mayer's reagent only produces a slight turbidity in

this solution the flour contains less than 0.1% of ergot.   If more is present a comparison with ergotinine solution of known strength may be made.   From this comparison the amount of ergot present is deduced by assuming it to have an alkaloidal content of 0.2%.

Other processes for the detection of ergot depend on the presence in ergot of a red acidic colouring matter (*sclererythrin*).   Hoffman's method (*Pharm. Zeit.*, **23**, 726, 742) consists in shaking 10 grm. of flour with 20 c.c. ether and 1 c.c. of 20% sulphuric acid for 1 hour; after filtering the filter is washed with ether until 25 c.c. total filtrate have been collected; 0.5 c.c. of a sodium hydrogen carbonate solution (1:14) is then added; this produces a violet colouration if more than 0.2% of ergot is present (comp. Adwujewski, *Pharm. Zeit. für Russl.*, 1894, **33**, 245).

Other papers on this subject are by Spaeth (*Pharm. Central-Halle*, 1896, **37**, 542), Lagerheim (*Zeit. Nahr. Genussm.*, 1902, 32) and Bernhart (*ibid.*, 1906, **12**, 321).

Alkaloids of the Calabar Bean.

Eserine or Physostigmine, $C_{13}H_{17}N_2(OH)(CO)(N.CH_3)$; or $C_{15}H_{21}O_2N_3$.   This base is the characteristic poisonous alkaloid of the Calabar bean (*Physostigma venenosum* or *P. faba*) of West Africa;[1] also known as the esere-nut, chap-nut, and ordeal bean.

Eserine forms crystals, which when pure are colourless, but which often have a pale red tint.   According to Petit and Polonovsky (*Bull. Soc. Chim.*, 1893, [iii], **9**, 1008), it melts at 105 to 106°.

Salway (*Trans.*, 1911, **99**, 2148) states that the alkaloid is dimorphous; he obtained stout prisms, melting at 86–87°, which recrystallised in the presence of a crystal of the more stable variety, melted at 105–106°.

Eserine is very slightly soluble in water, readily soluble in alcohol, ether, chloroform, benzene, and carbon disulphide; but insoluble in petroleum-ether.

Eserine is lævorotatory, the value for $[\alpha]_D$ being: in chloroform solu-

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[1] *Physostigma cylindrospermum* is probably occasionally substituted for the official bean. Salway (*Trans.*, 1911, **99**, 2150) percolated the finely powdered material with hot alcohol, and after evaporation extracted the residue with water.   The aqueous extract was made alkaline with sodium carbonate and shaken with ether.   After concentration the ethereal solution was shaken with successive portions of 5% sulphuric acid, until the extract became just acid.   To the neutral solution of physostigmine sulphate so obtained, a saturated solution of sodium salicylate was added, which precipitated physostigmine salicylate as an almost colourless, crystalline powder.

tion, $-75.8°$ to $-82°$; in 98% alcohol, $-89°$; and in benzene or toluene, $-120°$.

The aqueous solution of eserine is strongly alkaline to litmus, and precipitates ferric hydroxide from a solution of ferric chloride.

On exposure to air and light, an aqueous solution of eserine becomes red, and ultimately dark brown, with formation of a red, crystallisable colouring matter called *rubreserine*, $C_{13}H_{16}O_2N_2$, soluble in chloroform. The formation of rubreserine has been attributed to absorption of atmospheric ammonia, and is greatly facilitated by the presence of alkalies, even the traces dissolved from glass being said to be effective. On treating the reddened solution with hypophosphorous acid, sulphurous acid, hydrogen sulphide, sodium thiosulphate, or nascent hydrogen, in presence of a trace of free acid, the liquid is decolourised.

With alkali hydroxides and fixed alkaline carbonates, concentrated solutions of eserine salts give oily precipitates of the base, but no precipitates are formed in more dilute solutions. On shaking the alkaline liquid with air, it rapidly acquires a pink-red colour. On agitation with chloroform, rubreserine is dissolved out, and colours the chloroform orange-red.

If a drop of a very dilute solution of eserine be placed on a white plate, and brought in contact with a drop of dilute (5%) solution of alkali hydroxide, the point of contact will acquire a red colour. On evaporation the liquid becomes yellow, and the residue salmon-pink, and soluble in water with yellow colour.

If a minute quantity of eserine or one of its salts be treated with excess of ammonia, and the liquid heated on the water-bath, it turns in succession pale red, red, yellowish-red, yellow, green, and finally blue. If the liquid be evaporated to dryness, a blue or bluish residue is left, *eserine blue*, which according to Salway (*Trans.* 1912, **101**, 978) has the composition, $C_{17}H_{23}O_2N_3$. This is soluble in alcohol and crystalises in long prisms, capable of dyeing silk without a mordant, and staining the skin, etc. The colour dissolves in dilute acetic or other dilute acid with purple-red colouration, and the solution exhibits a strong reddish fluorescence when viewed by reflected light.[1] On evaporation, this liquid leaves a residue which is first green, but changes subsequently to blue, and is then soluble in alcohol, water, or chloroform, but not in ether. From the watery ammoniacal

[1] The ammonia test for eserine was first described in 1872 by Petit (*Journ. Pharm.*, 1871 [iv], **13**, 327), and is official in the French Codex and in the British and United States Pharmacopœias.

solution, chloroform only partially extracts the blue colouring matter. On treating the above-mentioned blue solutions with hydrogen sulphide or another reducing agent, they are first reddened and then decolourised. On heating the decolourised liquid on the water-bath, so as to expel the hydrogen sulphide, the blue colour returns.

All the foregoing colour-reactions appear to be due to the formation or decomposition of rubreserine. According to Eber (*Pharm. Zeit.*, 1888, 483), rubreserine is not a simple oxidation-product of eserine, a strongly alkaline volatile base being simultaneously formed, which, like rubreserine, has no action on the pupil. Neither rubreserine, the blue compound, nor the volatile base were found by Eber in the urine of an animal to which eserine had been administered; but a base was separated which closely resembled eserine except for the fact that it had no action on the pupil. The same inactive base was separated from certain samples of commercial eserine, and is stated by Eber to be formed by boiling a neutral solution of eserine. Eber believes this base to be an intermediate product in the formation of rubreserine from eserine, and attributes to its presence the very rapid reddening of some samples of commercial eserine. These results are confirmed by Ehrenberg, who finds that if alkalies be allowed to act on eserine in the absence of air and in the cold, a new base is formed, and can be obtained in crystals by means of dry ether. Ehrenberg calls this substance eseroline, and attributes to it the formula $C_{13}H_{18}ON_2$. On exposure to air it rapidly oxidises to rubreserine. It is not yielded by similar treatment of eseridine. For the properties and relationships of eseroline, rubreserine and eserine blue consult Salway (*Trans.*, 1912, **101**, 978).

If a solution of eserine or one of its salts be heated to boiling, and a few drops of strong nitric acid added, an orange-coloured liquid is obtained, which on addition of sodium hydroxide in excess yields an intensely violet solution, becoming wine-red on dilution with water. The violet colour is changed to pale orange by acids, and restored by alkalies (J. E. Saul, *Pharm. J.*, 1887, [iii], **17**, 642; 1893, [iii], **24**, 300).

Another form of the nitric acid test has been described by Ferreira da Silva (*Compt. Rend.*, 1893, **117**, 330). A minute fragment of eserine or one of its salts is treated in a porcelain capsule with a drop or two of fuming nitric acid. The clear yellow solution which is obtained changes to orange when heated to 100°, and on evaporation to dryness on the water-bath, while stirring, a green residue is obtained, for which da Silva proposes the name chloreserine. This dissolves in

water, or more readily in strong alcohol, with deep green colour, the colouring matter being recovered unchanged on evaporation. The residue dissolves in strong sulphuric acid to form a green, non-fluorescent solution. Ammonia does not affect chloreserine, but if a drop of nitric acid be allowed to fall on the green residue while on the water-bath, it is turned blue in those parts not directly in contact with the acid, and subsequently a reddish-violet solution is formed, which changes after a time to greenish-yellow. This solution, when diluted is fluorescent, appearing of a blood-red colour by reflected light, and greenish-yellow by transmitted light.

With strong sulphuric acid, solid eserine yields a yellow or brownish colouration, which changes gradually to orange or red. Concentrated hydrochloric acid also produces a pink or reddish colour when added to solid eserine, especially if the mixture be warmed.

Bromine-water, avoiding excess, gives an intense red colour with eserine, both in the solid state and in solution. Bleaching powder solution behaves somewhat similarly, but its action is difficult to control.

Except in very concentrated solution, eserine yields no precipitate with platinic or mercuric chloride, potassium dichromate. According to Salway a well-defined picrate is obtainable, crystallising from dilute alcohol in feathery needles, melting at 114° and only sparingly soluble in hot water, but readily so in alcohol. Phospho-molybdic acid and iodised potassium iodide precipitate eserine from very dilute solutions. With Mayer's reagent, eserine yields a precipitate of the composition $C_{15}H_{21}O_2N_3,HI,HgI_2$, melting at 70°, crystallising from alcohol in small prisms, and soluble in a mixture of alcohol and ether.

According to Eber, auric chloride, potassio-bismuth iodide, and potassio-zinc iodide, used as precipitants of eserine from a solution of the sulphate, give distinct reactions with quantities too minute for recognition by the physiological test.

According to the United States Pharmacopœia (8th Revision), the *Assay of Physostigma* seeds is carried out as follows: 20 grm. of the seeds in No. 60 powder are placed with 200 c.c. of ether in a conical flask and 10 c.c. of a 5% sodium hydrogen carbonate solution are added. The mixture is shaken at intervals and after 4 hours it is allowed to settle; 100 c.c. of ether (= 10 grm. of the drug) are decanted and acidified with N sulphuric acid. After adding 10 c.c. of distilled water and shaking, the aqueous layer is separated, and the ex-

traction is repeated by shaking with 2 c.c. N sulphuric acid $+$ 8 c.c. of water, and again with 1 c.c. N acid $+$ 9 c.c. of water. The three acid extracts are mixed, rendered alkaline with sodium hydrogen carbonate and shaken successively with 25, 20 and 15 c.c. of ether. The residue left on evaporation of these ethereal extracts is dissolved in 5 c.c. $N/10$ sulphuric acid and the solution, together with 20 c.c. of strictly neutral ether and 80 c.c. of distilled water, is placed in a stoppered bottle; 5 drops of iodeosin solution are added and the contents of the bottle are titrated with $N/50$ potassium hydroxide until, after shaking, the aqueous liquid is pink. 1 c.c. $N/10$ acid neutralises 0.0273 grm. of alkaloid. The United States Pharmacopœia demands a minimum alkaloidal content of 0.15% in the seeds of $P.$ $venenosum.$

Salway (*Trans.*, 1911, **99**, 2148) found that a sample when assayed by the above method yielded 0.091%, whereas 0.179% of alkaloid was actually isolated when working up the same material on a large scale. According to Salway three extractions with ether are quite insufficient to remove the alkaloid from sodium hydrogen carbonate solution.

Carr and Reynolds (*Pharm. J.*, 1908, [iv], 26, 542) found very great variation in the alkaloidal content of commercial specimens of Calabar beans, namely, between 0.04 and 0.27%.

Eserine derives its chief interest and practical importance from its energetic action on the pupil of the eye, which is powerfully contracted by very minute quantities of the alkaloid. Thus, if 0.010 grm. of eserine be dissolved in 10 c.c. of water, and one or two drops of this 0.1% solution be introduced into the eye, the pupil will become contracted to the size of a pin's head. The time required for this to occur will be not longer than fifteen minutes, but varies with different persons. Little or no inconvenience is occasioned, and the eye soon returns to its normal condition.

Administered internally, eserine acts as a powerful poison. The principal symptoms are usually severe griping pains, constant vomiting, and contracted pupils. Staggering and purging are also observed in some cases.

Eserine (in the free state, under the name of physostigmine) is official in the British Pharmacopœia, which describes it as occurring in colourless or pinkish crystals. The alkaloid is also official in the French Codex. In the United States Pharmacopœia, physostigmine sulphate and salicylate are official. *Physostigmine sulphate*, B_2,H_2SO_4, is described as a white or yellowish-white, microcrystalline powder,

very deliquescent, and gradually reddening on exposure to air and light. It melts at 150–151°, and dissolves very readily in alcohol and water, forming solutions neutral to litmus. *Physostigmine salicylate*, $B,C_7H_6O_3$, forms colourless or faintly yellowish shining needles, or short columnar crystals, soluble in 150 parts of cold water, or 30 of boiling water. It is also soluble in 12 parts of cold alcohol, and more readily on boiling. The salt melts at about 182–183°, has a faintly acid reaction to litmus, and acquires a reddish tint on exposure to light and air. *Physostigmine benzoate*, $B,C_7H_6O_2$, is prepared by mixing ethereal solutions of eserine and benzoic acid, and evaporating the ether, when the salt separates in short white prisms, m. p. 115 to 116°, which are not deliquescent, but dissolve in about 4 parts of cold water, and are also soluble in alcohol. *Physostigmine hydrobromide*, B,HBr, forms fibrous masses, non-deliquescent, and very soluble in water, melting at 224–226°.

Eseridine is a substance obtained by Eber (*Pharm. Zeit.*, 1888, 611) by the action of sulphurous acid on eserine, from which it is said to differ by the elements of water, having the formula $C_{15}H_{23}O_3N_3$. As a reducing agent is used in its preparation this composition is improbable. Eseridine has been prepared in the crystalline state by Boehringer, and has been proposed as a substitute for eserine in therapeutics, on the ground of its milder action. Its advantage over eserine is, however, denied by Schweber, the chief drawback to the use of either alkaloid being the ready susceptibility of the heart to its action.

Eseridine forms small crystals, melting at 132°, and evolving alkaline vapours when heated more strongly. The base itself is neutral to litmus.

Eseridine is readily soluble in chloroform, but only sparingly soluble in ether or alcohol.

In its chemical reactions eseridine closely resembles the parent alkaloid, the chief differences being the following: With ammonia, lime-water, or sodium hydroxide, eseridine becomes gradually yellow, and dirty-green, not red like eserine. On agitating a trace of eseridine with a minute particle of potassium iodate and water, a red solution is obtained, from which chloroform extracts a brownish-red colouring matter. In presence of acetic acid, the chloroform becomes violet from separation of iodine.

Salway could not obtain eseridine from calabar beans.

Eseramine, $C_{16}H_{25}O_3N_4$ (?) was isolated from calabar beans by

Ehrenberg (*Verh. Ges. Deut. Naturf. Aertzte*, 1893, [ii], 102, quoted by Salway). It forms colourless needles, melting at 238° and almost without physiological action. The same base (m. p. 245°) was obtained by Salway, in minute quantity (0.1 grm. from 123 kilo of beans).

Physovenine, $C_{14}H_{18}O_3N_2$, is another alkaloid present in calabar beans in extremely minute quantity. It was recently isolated by Salway from the ethereal mother-liquor of eseramine, and forms small colourless prisms, melting at 123°. Physovenine is, like physostigmine, very powerfully myotic. Salway regards it as an intermediate product in the conversion of physostigmine into eseroline referred to above; the change is represented by the following equations:

$$\underset{\text{Physostigmine}}{C_{15}H_{21}O_2N_3} + H_2O = \underset{\text{Physovenine}}{C_{14}H_{18}O_3N_2} + CH_3.NH_2$$
$$C_{14}H_{18}O_3N_2 = \underset{\text{Eseroline}}{C_{13}H_{18}ON_2} + CO_2$$
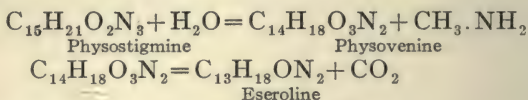
Alkaloids of Gelsemium.

The root-bark of yellow jasmine (*Gelsemium sempervirens*) contains the alkaloids gelsemine and gelseminine in company with gelsemic acid (page 34). The former alkaloid is crystallisable, and forms crystallisable salts, while gelseminine and its salts are amorphous. This is the nomenclature employed in England, but unfortunately much confusion has been caused by the use of these names in the opposite sense by most of the German investigators. Thus Spiegel, Goeldner, and Merck, in his market lists, call the crystalline alkaloid gelseminine. Brühl, in his *Pflanzenalkaloide* adheres to the English names. The total alkaloids of gelsemium root are stated to range from 0.15 to 0.25%, about three-fourths being gelsemine, though the medicinal activity of the tincture is mainly if not entirely due to the smaller amount of gelseminine.

For the extraction of the mixed alkaloids of gelsemium root, F. A. Thompson (*Pharm. J.*, 1887, [iii], **17,** 806) treats the finely powdered drug with about one-sixth of its weight of slaked lime, and exhausts the mixture with strong alcohol. The percolate is rendered faintly acid with dilute sulphuric acid, the calcium sulphate filtered off, and the filtrate evaporated to a syrup, which is allowed to cool, and water added as long as a precipitate is produced. On standing for 24 hours the liquid separates into two strata, the upper of which is chiefly gelsemic

acid, and the lower a solution of the sulphates of the alkaloids. This is drawn off, and the gelsemic acid washed with cold water. The solution is concentrated, and agitated with ether to remove a further portion of the gelsemic acid, the last portions being removed by similar treatment with chloroform. The aqueous liquid is then treated cautiously with sodium hydroxide and the liberated alkaloids extracted by agitation with chloroform. The chloroform solution is separated, and the alkaloids dissolved out by agitating with water acidified with sulphuric acid (not hydrochloric acid). From the dark solution of the sulphates thus obtained, the alkaloids are again liberated by alkali, shaken out with ether, and recovered from the separated ethereal solution by agitation with dilute hydrochloric acid. From this solution the sparingly soluble hydrochloride of gelsemine is deposited on standing, and may be purified by repeated crystallisation from hot alcohol, while the very soluble gelseminine salt remains dissolved.

An alternative method is to extract the gelsemium root with a mixture of 3 vols. of alcohol and one of ether, evaporate the solution, take up the residue with water, and precipitate the filtered liquid with lead acetate. The filtrate is freed from excess of lead by hydrogen sulphide and shaken with ether. The aqueous liquid is treated with alkali hydroxide, and the precipitated alkaloids extracted by agitation with ether.

For the extraction of **gelsemine,** Wormley recommends that the finely-powdered root should be exhausted with a mixture of equal measures of rectified spirit and water, and the clear liquid concentrated to a small bulk, allowed to stand for some time, and filtered. The filtrate is rendered slightly alkaline with ammonia, and agitated several times with ether. The ethereal solution, which contains both the gelsemine and the gelsemic acid, is treated with a slight excess of hydrochloric acid, added drop by drop, and allowed to stand for some hours. The alkaloid is precipitated as a more or less granular or crystalline hydrochloride, which adheres to the sides of the vessel. The ether is poured off, the precipitate washed with fresh ether, dissolved in a minimum of water, and the filtered solution treated with a slight excess of ammonia, which precipitates the gelsemine as a white curdy mass, which is rapidly filtered off, washed with a little cold water, and dried. On allowing the ammoniacal filtrate to evaporate spontaneously, prismatic crystals of gelsemine hydrochloride are deposited. The ethereal solution from which the gelsemine hydrochloride was

precipitated on evaporation leaves crystalline tufts of gelsemic acid, which may be washed with a little cold absolute alcohol, and recrystallised from boiling alcohol. From the dried root of *Gelsemium*, Wormley obtained, on an average, about 0.25% of gelsemine and 0.5 of gelsemic acid. He believes these principles to exist solely in the root-bark, and to be entirely absent from the woody portion.

Moore (*Trans.*, 1910, **97**, 2223) recently isolated gelsemine as follows: The powdered root was extracted with alcohol, and the resin left after evaporation of the alcohol was extracted with water. From the aqueous solution the gelsemic acid (*Scopoletin*) was removed by shaking with chloroform, and a little amorphous non-basic material was removed by amyl alcohol. The solution was then rendered alkaline with sodium carbonate and extracted with ether. On evaporation of the ether a pale yellow product remained, which readily crystallised from acetone and then consisted of pure gelsemine.

Many formulæ have at different times been proposed for *gelsemine*. (Sonnenschein, $C_{22}H_{38}O_4N_2$; Gerrard, $C_{24}H_{28}O_4N_2$; Thompson, $C_{54}H_{69}O_{12}N_4$; Cushny, $C_{49}H_{63}O_{14}N_5$; Spiegel, $C_{22}H_{26}O_3N_2$; Goeldner, $C_{22}H_{26}O_3N_2$.) Quite recently Moore (*Trans.*, 1910, **97**, 2230) claims to have definitely established the formula $C_{20}H_{22}O_2N_2$ for gelsemine, dried at 120° until constant. His analyses are in good agreement with this formula and as the melting-point of his preparation was considerably higher than that given previously, we may consider the claim justified. According to Moore, gelsemine forms pale yellow glistening prisms, m. p. 178° (Goeldner gives 160°). It crystallises from acetone with one mol. of the solvent. The alkaloid is dextrorotatory; a 1.55% aqueous solution giving $[\alpha]_D = +2.6°$ and a 2.033% solution of the anhydrous base in chloroform giving $[\alpha]_D = +15.9°$.

Gelsemine is sparingly soluble in water (1:644), more readily in alcohol, and very easily in ether and chloroform. It has a strong alkaline reaction, a persistent and very bitter taste, dilates the pupil, and is poisonous.

Gelsemine forms a series of well-defined and crystallisable salts. The *hydrochloride* $C_{20}H_{22}O_2N_2$,HCl forms sparingly soluble prisms melting indefinitely at or above 310°. The nitrate forms glistening prisms, m. p. above 280° (Moore).

Gelsemine shows some interesting colour-reactions. Strong nitric acid dissolves the pure alkaloid with little or no colour, but on allowing

the liquid to evaporate spontaneously on porcelain, a permanent bluish-green colour is obtained, even when only a very minute trace of gelsemine is present. As usually obtained, gelsemine residues yield with nitric acid yellowish or brownish-green colourations, rapidly changing to deep green.

Pure gelsemine dissolves in strong sulphuric acid without colouration, even on warming; but if not perfectly pure, a more or less reddish or brownish colour is obtained, which gradually becomes pinkish, and on heating acquires a chocolate or purple tint.

When solid gelsemine, or one of its salts, is treated with strong sulphuric acid and an oxidising agent (e.g., potassium dichromate or ferricyanide, manganese dioxide, etc.), in the manner employed in testing for strychnine, a fine reddish-purple or cherry-red colouration is produced, rapidly changing to a bluish-green or blue tint. Wormley states that 0.0001 grain will respond to this test, and that even 1/10 of this quantity may produce the reddish-purple colouration. The foregoing colour-test fails if the mixture of alkaloid with sulphuric acid be heated before adding the oxidising agent; but Wormley states that the alkaloid is not destroyed thereby, since it may be recovered by rendering the solution alkaline, and extracting with ether. The behaviour of gelsemine with the oxidising mixture somewhat resembles that of strychnine, but differs therefrom in the order in which the tints appear (compare Strychnine, Vol. 6).[1]

Gelsemine gives precipitates with most of the general reagents for alkaloids, but they are not very characteristic or highly insoluble. Iodised potassium iodide gives a brown amorphous precipitate in a solution of 1 in 10,000.

In frogs, gelsemine produces tetanic convulsions, followed by paralysis of the extremities of the motor nerves. On rabbits its poisonous effects are not marked.

Gelseminine (Merck's "amorphous gelseminine") is an alkaloid of doubtful formula occurring with gelsemine. It is described by L. Spiegel (Ber., 1893, 26, 1054), as a white amorphous powder which softens at 105° and melts at 120°, with partial decomposition.

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[1] J. B. Nagelvoort finds the distinction between gelsemine and strychnine to be sharpest when the oxidising reagent is a freshly-made mixture of 16 c.c. of concentrated sulphuric acid with 10 c.c. of water containing in solution 0.020 grm. of potassium permanganate. Strychnine and its salts and solutions, when dropped into this mixture, give an intense *blue* colouration, rapidly changing to purple, and finally a permanent cherry-red; while gelsemine and its preparations never give a blue tint, but always and immediately a cherry-red, which reaction fades to colourless in less than a minute. 0.002 grm. of a gelsemine or gelseminine salt, dissolved in 2 c.c. of water, and added to 3 c.c. of the oxidising mixture gives the reaction perfectly.

Gelseminine is intensely bitter, and exhibits a strong alkaline re-action. It is insoluble in water, but soluble in alcohol, ether, and chloroform.

The salts of gelseminine are soluble, amorphous, yellowish bodies.

With dilute nitric acid gelseminine yields brown indefinite com-pounds, together with an acid of the formula $C_{17}H_{20}O_8N_3$. This compound is deposited from alcohol in pale yellow crystals, which darken without melting when heated to $350°$. With strong nitric acid, gelseminine gives a green, and with sulphuric acid a yellow colouration, changing on addition of oxidising agents to violet, and finally to green.

Gelseminine dilates the pupil, and acts as a powerful poison, pro-ducing respiratory failure in rabbits as well as in frogs. o.oo1 grm. of the alkaloid proved fatal to a rabbit, whereas o.5 grm. of gelsemine was without effect. It is very probably to the gelseminine (perhaps modified by the co-occurring resinous bodies) that the action of gel-semium tincture is due.

A method of separating gelsemine from gelseminine, for which F. A. Thompson claims priority (*Pharm. J.*, 1887, [iii], **17,** 806), is based on the solubility of the hydrochloride of the latter base in an equal weight of water, whereas gelsemine hydrochloride is but spar-ingly soluble (1:39).

The **gelsemic acid** of Wormley has been shown by E. Schmidt (*Arch. Pharm.*, 1898, **236,** 236) to be a monomethylether of æsculetin. The latter substance is 4:5-dihydroxycoumarin.

$$
\begin{array}{c}
\text{CH} \\
\text{HO}\diagup\diagdown\text{CH} \\
\text{HO}\diagdown\diagup\text{CO} \\
\text{O}
\end{array}
$$
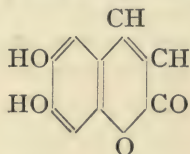
Gelsemic acid has the formula $C_{10}H_8O_4$; it is not known which of the two phenol hydroxyls of æsculetin is methylated. Moore (*loc. cit.*) prefers the name *Scopoletin*, as gelsemic acid is identical with the latter substance isolated from *Scopolia japonica* by Eykman (*Rec. trav. chim.*, 1884, **3,** 171).

According to Moore scopoletin (from gelsemium) after recrystal-lisation from alcohol forms long, almost colourless needles, m. p. 204°. It is soluble in alkalies to a liquid having an intense yellow colour and greenish-blue fluorescence, perceptible even in very dilute solutions (1:100,000).

The fluorescence is readily destroyed by free acids, which fact distinguishes it from the fluorescence produced by quinine.

Nitric acid dissolves gelsemic acid with yellow or orange colour, which is changed by excess of ammonia to a permanent blood-red tint. The reaction is very delicate, and is also produced by æsculin.

Gelsemic acid dissolves slowly in strong sulphuric acid with yellow or reddish colour, not materially changed by warming, if the pure substance be used. On adding potassium dichromate, reduction, with green colouration, ensues. If a drop of ammonia be added to a drop of solution of gelsemic acid in strong sulphuric acid, placed on a slip of glass, gelsemic acid immediately separates at the junction of the two drops in crystalline needles of a characteristic microscopic appearance. Wormley describes this reaction of gelsemic acid as one of the most delicate and characteristic known, and as not readily interfered with by the presence of foreign matter, with the additional advantage of not being simulated by æsculin.

Gelsemic acid (scopoletin) further differs from æsculin in being somewhat soluble in chloroform (1:230 at 0°) and in being neutral to litmus. Æsculin is insoluble in chloroform and acid to litmus.

Gelsemic acid is not known to possess any active medicinal properties, but its reactions, and especially its fluorescence in alkaline solutions, are of service as a test for preparations of gelsemium.

Alkaloids of Peganum Harmala.

Peganum Harmala is a herbaceous plant, growing wild in Southern Europe, particularly in Southern Russia. The seeds contain about 4% of alkaloids, almost exclusively in the seed-coat. Two-thirds of the alkaloidal content is *harmaline*. The alkaloids are obtained by extracting the powdered seeds with acidified water and precipitating the salts as hydrochlorides by saturating the aqueous extract with common salt. In order to ensure complete precipitation the excess of free acid in the extract should be as small as possible. The crude hydrochlorides are dissolved in water and the solution is treated with charcoal and warmed to 50 to 60°; ammonia is then added which first precipitates *harmine*. An excess of ammonia afterward precipitates *harmaline*.

Harmine, $C_{13}H_{12}ON_2$, crystallising in prisms, m. p. 256 to 257°, is optically inactive and partially sublimes unchanged. It is sparingly

soluble in water, alcohol and ether. Its solutions show a blue fluorescence (Fischer and Koerner, *Ber.*, 1885, **18,** 401).

Harmaline, $C_{13}H_{14}ON_2$, is *dihydroharmine* and crystallises from methyl alcohol in pale yellow leaflets, m. p. 238°. The base itself is so little soluble in water that it is almost tasteless; the soluble salts are bitter. The base is readily soluble in hot alcohol and is precipitated from its alcoholic solution by ether. The *hydrochloride* $C_{13}H_{14}ON_2,HCl,H_2O$ forms yellow needles. For the constitution of harmine and harmaline, see Perkin and Robinson (*Trans.*, 1912, **101,** 1775).

The physiological properties of harmaline have been investigated by Tappeiner and Neuner (*Arch. exp. Path. Pharm.*, 1895, **35,** 69) who stated that it is a convulsant poison. More recently Flury (*ibid.*, 1910, **64,** 105) has confirmed this; he finds that it is also an emmenagogue and has diaphoretic and anthelmintic properties. It is also a narcotic, which explains the use of *Peganum harmala* for the same purposes as Indian hemp, *Cannabis indica*. The anthelmintic properties are somewhat marked; *Ascaris* worms are killed in a 1 : 25,000 solution of harmaline.

Alkaloid of Barley Germs (Hordenine).

Léger (*Compt. Rend.*, 1906, **142,** 108) isolated a base from barley germs, having the composition $C_{10}H_{15}ON$ which he named *hordenine*. Later (*Compt. Rend.*, 1906, **143,** 234, 916) he assigned to it the constitution:

$$\text{OH} \left\langle \underline{\quad} \right\rangle \text{CH}_2.\text{CH}_2.\text{N}(\text{CH}_3)_2.$$
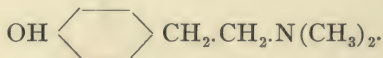
The base is therefore hardly a typical alkaloid. The above constitutional formula was confirmed by Gaebel (*Arch. Pharm.*, 1906, **244,** 435). More recently Barger (*Trans.*, 1909, **95,** 2193) has synthesised hordenine from phenylethyldimethylamine by nitration, reduction of the *p*-nitro derivative and diazotisation of the resulting amino-derivative. Rosenmund (*Ber.*, 1910, **43,** 306) also obtained it synthetically from its methyl-ether (prepared from anisaldehyde).

Hordenine is obtained from malt (or better from malt germs) by extraction with 96% alcohol. After evaporation of the alcohol, the syrupy residue is dissolved in water, acidified, washed with ether, made alkaline with sodium carbonate and thoroughly extracted with ether. The crude hordenine remains behind on evaporation of the ether and can

be purified by recrystallisation from dry ether or from benzene with
the aid of animal charcoal, or more conveniently by distillation under
greatly reduced pressure.

Hordenine forms colourless crystals, m. p. 117.5°, b. p. 173 to 174°
under a pressure of 11 mm. The base is readily soluble in alcohol and
in hot water, less so in cold water and in ether, very little in cold ben-
zene. Like tyrosine, hordenine gives an intense red colouration when
boiled with Millon's reagent; it reddens phenolphthaleïn, is not col-
oured by concentrated sulphuric acid, and reduces acid potassium per-
manganate in the cold and ammoniacal silver nitrate on warming.
The phenolic character of the base prevents its extraction by ether
from a solution in sodium hydroxide. The tertiary nature is shown by
the production of a methiodide with methyl iodide. A number of
crystalline salts and other derivatives of hordenine have been de-
scribed (Léger, *Compt. Rend.*, 1907, **144,** 208). The physiological
properties of hordenine have been investigated by Camus (*Arch. int.
de Pharmacodyn. et de Thér.*, 1906, **16,** 43). The base has a compara-
tively slight action in raising the blood pressure of mammals, on
intravenous injection; the action is much feebler than that of
the parent base p-hydroxyphenylethylamine. The therapeutic ad-
ministration of hordenine by the mouth in dysentery and similar
diseases appears to be entirely empirical and was suggested by
the use of malt in such cases. The alkaloid is hardly toxic; the dan-
gerous dose when given by the mouth is in man 60 grm. (Sabrazès and
Guèrive, *Compt. Rend.*, 1908, **147,** 1076). Hordenine methiodide,
when injected intravenously, has a powerful transitory effect in raising
the blood pressure; the action superficially resembles that of adrenaline,
but is in reality of the nicotine type.

Alkaloids of Ipecacuanha.

The roots of *Cephaëlis Ipecacuanha* and *C. acuminata*[1] have been
long known to contain an alkaloidal principle having violent emetic
properties, and hence called emetine. Owing to the amorphous
character of the alkaloid and its salts, as ordinarily prepared, and
their unstable character, the preparation of the pure base presents
great difficulties, and hence very discordant formulæ and characters
have been assigned to the alkaloid. Pure emetine appears to have

[1] The commercial varieties of ipecacuanha have been described by E. M. Holmes (*Year-
book Pharm.*, 1893).

been first prepared by Glénard (*Ann. Chim. Phys.*, 1876, [v], **8,** 233), who ascribed to it the formula $C_{15}H_{22}O_2N$. In 1893, Paul and Cownley (*Pharm. J.*, 1893, [iii], **24,** 61) announced the isolation of a second alkaloid from ipecacuanha, which alkaloid they called cephaëline, and this they have since proved to co-exist with emetine in the root from various sources. The following description of the alkaloids of ipecacuanha is taken from subsequent papers by Paul and Cownley, which also contain an interesting historical account of previous researches (*Pharm. J.*, 1894, [iii], **25,** 111, 373, 690).

In the examination of Brazilian ipecacuanha (*C. Ipecacuanha*), a quantity of the drug was extracted with alcohol, the liquor mixed with basic lead acetate until no further precipitate was formed, the filtered liquid evaporated to dryness, and the residue dissolved in weak acid. The filtered liquid was mixed with ether, ammonia added in slight excess, and the whole shaken. The ethereal solution of the alkaloids was separated, shaken with dilute sulphuric acid, and the acid liquid treated with excess of sodium hydroxide, which precipitated the emetine, while retaining the cephaëline in solution. The precipitate of crude emetine thus obtained, after washing and drying, amounted to 1.34% of the root used. To purify the emetine it was dissolved in dilute acid, and the solution shaken with sodium hydroxide in presence of ether, this operation being repeated until the cephaëline had been completely separated. The insoluble emetine was then converted into hydrochloride, the salt recrystallised from water, and the base finally precipitated by ammonia. The caustic alkaline solution resulting from the above treatment was neutralised, ammonia added, and the cephaëline extracted by agitation with ether, which on evaporation left 0.6% on the weight of the root.

In the examination of New Granada ipecacuanha (*C. acuminata*), the powdered drug was mixed with lime and extracted with amyl alcohol, the subsequent separation of the extracted bases being effected as in the previous case.

Both emetine and cephaëline are present in Brazilian ipecacuanha, and also in that from Carthagena, New Granada. In the drug from the latter source, cephaëline appears generally to predominate over emetine, and probably that is also the case in the stalky portion of the Brazilian drug.

The following are the characters ascribed by Paul and Cownley to emetine and cephaëline prepared in the foregoing manner:

Emetine, $C_{30}H_{44}O_4N_2$,[1] is a nearly colourless base, and apparently uncrystallisable. It melts at about 68°, and rapidly acquires a yellowish colour on exposure to light. It is only slightly soluble in water, but dissolves readily in alcohol, ether, chloroform, and benzene, though only very sparingly soluble in petroleum-spirit, even when hot, as it melts to a resinous mass not readily acted on. The solutions become coloured on exposure to light, and give a reddish deposit. On evaporation of any of these solutions, the emetine is left as a transparent varnish, which is strongly alkaline to litmus and neutralises acids completely.

When precipitated from the solution of one of its salts by alkali hydroxide, emetine is insoluble in excess of the reagents.

The *sulphate*, *acetate*, and *oxalate* of emetine are very soluble in water and alcohol, and apparently uncrystallisable. The *hydro-chloride*, $B,2HCl$, may be obtained in a crystalline condition by evaporating its aqueous solution slowly, or by adding ether to its alcoholic solution. It is nearly insoluble in excess of hydrochloric acid, which converts the base into a mass of silky crystals of $B,2HCl$. A 5% solution of the hydrochloride, mixed with potassium bromide or iodide, gives a dense precipitate, which dissolves on addition of alcohol, the solution yielding tufts of silky needles of the *hydrobromide* or *hydriodide* of the base when slowly evaporated. The sparing solubility of the former salt has been utilized in preparing the alkaloid from the drug. —German patent No. 99090. It crystallises with $2H_2O$ and melts at 264°.

Emetine nitrate is very sparingly soluble in water, and separates as a resinous mass on adding potassium nitrate to a 5% solution of the hydrochloride. It dissolves more readily in alcohol, and by gradually adding ether to the solution the salt separates in crystalline tufts. B,H_2PtCl_6 is a buff-coloured, amorphous precipitate, almost insoluble in water or alcohol.

According to Kunz-Krause (*Arch. Scienc. Phys. nat. Genève*, 1895, [iii], **34**, 290) emetine is a diacid tertiary diamine, uniting readily with methyl iodide and containing four methoxyl groups. It is probably a quinoline derivative.

Cephaëline, $C_{28}H_{40}O_4N_2$, appears to be the lower homologue of

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[1] Paul and Cownley first gave the formula $C_{15}H_{22}O_2N$, but afterward induced Hesse to carry out molecular weight determinations, which led to the adoption of the double formula for both alkaloids. The percentage composition found by Paul and Cownley has been confirmed by R. A. Cripps (*Pharm. J.*, 1895, [iv], **1**, 160) and especially by G. Frerichs and N. de Fuentes Tapis (*Archiv Pharm.*, 1902, **240**, 390).

emetine. When precipitated from a solution of one of its salts by ammonia, it is colourless, but rapidly becomes yellow on exposure to light. The ammonia precipitate melts at about 102°. By evaporating a solution in alcohol or ether the base is left as a faintly yellow transparent varnish, but in a closed vessel a concentrated ethereal solution gradually deposits bunches of delicate silky needles, which melt at 88° and after drying at 80° melt at 119 to 120°. (Frerichs and de Fuentes Tapis).

Cephaëline is very much less soluble in ether, but more soluble in petroleum-spirit than emetine, from which it is sharply distinguished by dissolving in solutions of the alkali hydroxide. With the exception of the *hydrochloride*, which is deposited in transparent rhombic crystals from a solution containing excess of hydrochloric acid, salts of cephaëline are apparently uncrystallisable; but they otherwise much resemble the corresponding salts of emetine. $C_{28}H_{40}N_2O_4,H_2PtCl_6$ is of a more pronounced yellow than the corresponding salt of emetine.

The physiological action of emetine and cephaëline appears to be very similar. Both alkaloids act as emetics in doses of about one-sixth of a grain, causing at the same time a feeling of considerable depression.

Except the foregoing, all descriptions of the characters and reactions of emetine have reference to indefinite mixtures of true emetine with cephaëline, as obtained from ipecacuanha. It is probable that other alkaloids are present in some cases, and such substances have been actually obtained. Thus Cripps and Whitby (*Year-book Pharm.*, 1891, 390) obtained from ipecacuanha a small quantity of alkaloid, which was extracted by chloroform but not by ether from its alkaline solutions. It gave faintly most of the colour-reactions of emetine, but appeared to contain some other substance in addition. Cripps and Whitby also obtained an alkaloid, not extracted from the root by rectified spirit, unless previously set free from its natural combination by means of an alkali, but which could be readily extracted by water or dilute acid. This substance gave a brick-red colouration with nitric acid; a brownish-purple with sulpho-molybdic acid, not becoming blue on adding hydrochloric acid; a reddish-brown with hydrochloric acid alone; a faint emetine reaction with bleaching powder, and a yellow precipitate with Mayer's solution.

Paul and Cownley (*Pharm. J.*, 1895, [iii], **25,** 690) announced the isolation of a third alkaloid from ipecacuanha, not improbably identical

with that previously obtained by Cripps and Whitby. It exists in the drug in very small quantity (0.04 to 0.06%), has apparently a much higher molecular weight than these alkaloids, and differs from them in being very sparingly soluble in ether. It is soluble in alkaline liquids, and hence remains in the mother-liquor from which emetine and cephaëline have been extracted by agitation with ether. It is dissolved on shaking this liquid with chloroform. As obtained by slow evaporation of its ethereal solution, this alkaloid is obtained in transparent, lemon-yellow prisms, which melt at 138°, neutralise acids, and dissolve readily in alcohol and chloroform, to solutions which darken on exposure to light, and deposit a brown substance. Later Paul and Cownley suggested the name *psychotrine* for this alkaloid. According to Lewin it has no emetic properties.

Arndt (*Year-book Pharm.*, 1889, 136) obtained from ipecacuanha root from 0.3 to 0.5% of an ammonium base which yielded trimethyl-amine when distilled with concentrated alkali. Later Arndt indentified the base as choline, which exists in the root as an insoluble tannate. To this Arndt attributes the high results obtained when the assay of ipecacuanha is conducted by exhausting the root with acid solvents.

For the preparation of the mixed alkaloids (emetine and cephaëline of Paul and Cownley) from ipecacuanha, R. A Cripps recommends that the powdered root should be exhausted by percolation with cold alcohol (60° O. P.), and the alcoholic liquid distilled under reduced pressure. To the extract so obtained dilute hydrochloric acid is added in slight excess, and the liquid filtered. The filtrate is treated with a solution of sodium carbonate, and the precipitated alkaloids collected on a filter, washed slightly, and dried in the dark over strong sulphuric acid. The product is finely powdered and boiled with ether (sp. gr. 0.720), the ethereal solution filtered, and dry hydrogen chloride gas passed through the filtrate to saturation. The alkaloids are precipi-tated as hydrochlorides, which may be filtered off, dissolved in water, and the alkaloids recovered by precipitation with sodium carbonate, washed with water, and dried over sulphuric acid.

Thus prepared, the mixed alkaloids form a whitish amorphous powder, which rapidly becomes yellow and then brown on exposure to light, and dissolves in alcohol to a yellowish solution. It is very slightly soluble in cold water (1:1000), but somewhat more readily on heating. Alcohol, methyl alcohol, amyl alcohol, chloroform, ether,

and benzene dissolve the alkaloids readily, the last three solvents extracting them from aqueous liquids in presence of ammonia. Petroleum-spirit dissolves only traces of the alkaloids in the cold, but takes up a larger quantity on boiling. The mixed alkaloids are also soluble in carbon disulphide, ethyl acetate, turpentine, etc. The solutions of ipecacuanha alkaloids are optically inactive and strongly alkaline to litmus.

When heated, the alkaloids fuse to a nearly colourless liquid, which gradually becomes yellow or brown. The m. p. is variously stated from 50 to 74°, and depends upon the relative proportions of emetine and cephaëline present. The alkaloids of ipecacuanha are very bitter and highly poisonous, being violent emetics and depressants. The medicinal dose ranges from 1/100 to 1/25 grain as an expectorant, or 1/5 to 2/5 grain as an emetic.

The most characteristic of the reactions of the ipecacuanha alkaloids is that with bleaching powder. If a drop of solution of bleaching powder be applied to a fragment of the solid substance or to one of its salts, and a drop of acetic acid then added, a very persistent bright orange or lemon-yellow colour is produced. If the solution of bleaching powder be added to a solution of the alkaloids in dilute hydrochloric acid, an orange colouration is produced and a yellow precipitate formed.

R. A. Cripps and Whitby (*Year-book Pharm.*, 1891, 390) enumerate the following additional reactions of the ipecacuanha alkaloids soluble in ether. Few of the reactions are characteristic, the best being that with sulphomolybdic acid:

With the solid alkaloids: Sulphuric acid, very pale yellow, becoming pale brown when heated to 100°; sulphuric acid and sugar, faint pinkish-brown, becoming salmon-pink; sulphomolybdic acid, pale yellowish-pink becoming greenish, and on adding hydrochloric acid greenish-blue, changing to rose with green at the edges; hydrochloric acid alone, no colouration; nitric acid, pale orange-brown, changing to bright orange-red.

With a solution in dilute hydrochloric acid: Auric chloride, yellow precipitate, soluble on warming; platinic chloride, pale yellow precipitate soluble in spirit; phospho-tungstic acid, white precipitate; Sonnenschein's solution, yellow; potassio-bismuth iodide, brilliant orange-red precipitate; potassio-cadmium iodide, white; Mayer's solution, white; and iodised potassium iodide, bright brown.

The colour reactions of the alkaloids of ipecacuanha have formed the subject of a paper by A. H. Allen and G. E. Scott (*Pharm. J.*, 1902, [iv], **15**, 552). The immediate formation of Prussian blue, in a solution of potassium ferricyanide containing ferric chloride, is said to be particularly characteristic of cephaëline, and therefore of the mixed alkaloids, distinguishing them from opium alkaloids.

The alkaloids of ipecacuanha neutralise acids perfectly, and their amount can be ascertained by titration with methyl-orange or iodeosin. The plan can be conveniently applied to the alkaloids shaken out by ether or chloroform.

When in a nearly pure state ipecacuanha alkaloids may also be determined with tolerable accuracy by titration with Mayer's solution, if certain precautions be taken.[1]

Ipecacuanha Root.—At present only the root of Brazilian ipecacuanha is official, and as there is some inducement to substitute other varieties, it is important to distinguish these from the official kind. According to H. G. Greenish (*Pharm. J.*, 1895, [iv], **1**, 137) this may be effected by a very careful microscopic examination of the specimen.[2] Thus, ipecacuanha *root*, whether Brazilian or Carthagena, may be distinguished as such in the form of powder by (a) the shape and size of the starch grains, (b) the absence of vessels, presence of perforated tracheids, (c) the acicular raphides, and (d) the emetine reaction with chlorine. The *stem* may be distinguished from the root (in powder) by (a) the presence of sclerenchymatous cells, (b) of lignified cells of the pith, and (c) of spiral vessels. *Carthagena* ipecacuanha may, in most cases, be distinguished from *Brazilian* by the larger size of its starch grains. In this respect it must be remembered that Carthagena roots with small starch grains occur that are practically indistinguish-

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[1] To obtain fairly accurate results, the liquid must contain approximately 1 part of the alkaloid in 500 (Jones, *Year-book Pharm.*, 1886, 543), and the addition of Mayer's solution should be continued until a few drops of the filtrate show the same degree of opalescence by addition of more Mayer's solution or of alkaloidal solution.
[2] An examination by Greenish of 32 samples of powdered ipecacuanha obtained by purchase from retail pharmacists, showed the absence of any adulterant in the shape of foreign starch or other drugs. Twelve of the 32 samples proved to be the Carthagena (New Granada) root, and possibly a few of the 20 classed as Brazilian may have been Carthagena or of mixed origin. Only 1 of the samples could claim to be really good Brazilian root free from stem, although this is an impurity which can be readily removed from the commercial drug by picking. Of the samples examined, Greenish classes 22% as good Brazilian, 31 as medium, 10 as bad, and 37% as Carthagenian. Ranwez and Campin (*Annal. de Pharm.*, **1**, 114, 238) classified the ipecacuanha of Belgian commerce (Louvain) as follows: Normal Brazilian, 15%; too woody, 40; Carthagena (wholly or partly), 15; and false cultivated root (wholly or partly), 30%. The last description is probably intended to apply to the drug offered at the London sales as "East Indian root," probably the rhizome of *Cryptocoryne spiralis*. This has the characteristics of a monocotyledonous rhizome, does not possess the odour of true ipecacuanha, and contains no emetine. The external appearance, microscopic characters, and chemical reactions render it easy to distinguish the drug from true ipecacuanha root.

able, when powdered, from Brazilian roots with large grains.   (See also H. G. Greenish, *Pharm. J.*, 1895, [iii], **25,** 685.)

The assay of Ipecacuanha has received much attention, and many different processes have been devised for the purpose.   Generally, the aim of investigators seems to have been to obtain the total alkaloids without distinction of emetine from the others.   Many of the processes proposed are invalidated by the readiness with which emetine undergoes decomposition.   Zinoffski and Dragendorff applied the reaction with Mayer's solution to a hydro-alcoholic extract of the root, after removing alcohol by evaporation, but this process has been shown to give results above the truth.[1]   Flückiger, followed by Jones and Ransom, extracted the root with boiling ammoniated chloroform, the last-named dissolving the residue obtained on evaporating this solution in acidified water and titrating with Mayer's solution.   The various processes for the assay of ipecacuanha published prior to 1889 were reviewed by Cripps and Whitby (*Pharm. J.*, 1889, [iii], **19,** 721), who recommended the use of ethyl acetate as a solvent; but Cripps, in a more recent paper (*Pharm. J.*, 1895, [iii], **25,** 1093), gives preference to the following modification of Lyons' process (*Pharm. J.*, 1886, [iii], **16,** 627).

Place in a small flask (capacity about 50 c.c.) 2.5 grm. of ipecacuanha in fine powder, and weigh the flask, cork, and contents.   Nearly fill the flask with a mixture of ether (sp. gr. 0.720) 250 parts, ammonia 10 parts, and alcohol 20 parts, and set aside, shaking occasionally, for twenty-four hours.   Now weigh the flask and its contents before removing the cork, decant rapidly as much as possible of the clear liquid, cork the flask, and reweigh.   The alkaloids may now be separated from the ethereal solution by repeated agitation with dilute acid, and again washing out from the acid liquid by ether, followed by chloroform, after rendering alkaline with ammonia or sodium carbonate. The alkaloidal solution is evaporated in a current of air, and the residue dried at a temperature not exceeding 60°, weighed, and finally dissolved in 5 c.c. of $N/20$ hydrochloric acid and titrated back with $N/100$ sodium hydroxide, using iodeosin as an indicator.   1 c.c.= 0.00254 grm. emetine (or 0.00248 grm., if Paul's formula be taken). The calculations need no explanation, but may be much simplified and several weighings avoided if the extraction be carried out in a small cylindrical percolator, the diameter of which (for 2.5 grm.) should be 11 to 12 mm.

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[1] See *Pharm. J.*, 1889, [iii], **19,** 723.

C. C. Keller (*Year-book Pharm.*, 1894, 127) has described the following method of assaying ipecacuanha. It has the merits of being tolerably simple, and of yielding results which agree fairly with those obtained by Lyons' process. A weight of 12 grm. of the air-dry drug is shaken in a dry bottle for 5 minutes with 90 grm. of ether and 30 of chloroform. 10 c.c. of ammonia is next added, and after half an hour 10 c.c. of water. 100 c.c. measure of the clear liquid is then poured off, and shaken in a separator with 25 c.c. of 1% hydrochloric acid. The acid liquid is run off, and the shaking repeated with 15 c.c. and with 10 c.c. respectively of the same dilute acid. The acid liquid is then made alkaline with ammonia, and agitated twice with a mixture of 3 parts of chloroform with 2 of ether. The solvent is then separated, evaporated, and the alkaloidal residue weighed or titrated with $N/10$ hydrochloric acid. Instead of weighing the residue, it is in some respects preferable to titrate it with standard hydrochloric acid. As an indicator methyl-orange may be used, but the end-point is not very sharply marked (T. P. Blunt, *Pharm. J.*, 1890, [iii], **20,** 809). Logwood tincture is preferable. It is a good plan to dissolve the alkaloids in a moderate excess of $N/10$ hydrochloric acid, and titrate back with $N/20$ sodium hydroxide. Keller states that 1 c.c. of $N/10$ hydrochloric acid neutralises 0.0254 grm. of emetine, which it would if the formula of neutral emetine hydrochloride were $C_{30}H_{40}O_5N_2,2HCl$, as contended by Kunz-Krause. If $C_{30}H_{44}O_4N_2$, the formula of Paul and Cownley, be adopted as correct, the weight of emetine combining with 1 c.c. of $N/10$ acid will be 0.0248 grm.; or 0.0241 grm. of a mixture of equivalent proportions of emetine with cephaëline.

The Assay of Ipecacuanha has been dealt with by G. Frerichs and N. de Fuentes Tapis (*Arch. Pharm.*, 1902, **240,** 390, where a full account is also given of assay methods suggested by others). By way of preliminary, these authors analysed both bases and their salts, and confirmed the formula $C_{30}H_{44}O_4N_2$ and $C_{28}H_{40}O_4N_2$ previously assigned by Paul and Cownley to emetine and cephaëline respectively. Psychotrine is soluble in chloroform, but not in ether. As it is not an emetic, and only present in small quantity, it is most convenient to neglect it in the assay, so that the much more convenient ether can be used instead of chloroform. The following modification of Keller's process is recommended: 6 grm. of the finely powdered root is shaken with 60 grm. of ether, 5 c.c. of ammonia (or sodium carbonate solution). The mixture is left standing for an hour, with occasional shaking; 10 c.c.

of water is then added and after thorough agitation 50 c.c. of ether are filtered off. Half the ether is evaporated on the water bath and the ethereal solution, thus concentrated, is extracted with 10 c.c. $N/10$ hydrochloric acid. The ether is next washed twice with 10 c.c. of water; the acid extract and washings are filtered into a flask and made up to 100 c.c. with water. Enough ether is added to give, after shaking, a layer of 1 cm. thickness. After adding 5 drops of a 1 in 250 solution of iodeosin, the mixture is titrated with $N/10$ sodium hydroxide. The number of c.c. of alkali required, multiplied by 0.024, gives the quantity of emetine and cephaëline in 5 grm. of the root, or the same number multiplied by 0.482 gives the percentage.

The estimation may also be carried out gravimetrically. For this purpose the above-mentioned acid extract is rendered alkaline with ammonia and the aqueous solution is shaken with 50 c.c. of ether. 40 c.c. of the ethereal solution are then evaporated and the residue is dried at 100°, giving the amount of the two alkaloids in 4 grm. of the drug. If desired, the emetine may be separated by utilising the solubility of the cephaëline in sodium hydroxide, from which it is not shaken out by ether. The latter alkaloid dissolves in Fröhde's reagent without much change of colour, but the solution is at once coloured a deep blue on the addition of a trace of a chloride, or of hydrochloric acid. Emetine is not coloured under these conditions.

The United States Pharmacopœia (8th Revision) gives the following assay method for Ipecacuanha: 15 grm. of the root in No. 80 powder is shaken for 5 minutes in a 250 c.c. conical flask with a mixture of 115 c.c. of ether and 35 c.c. of chloroform. 3 c.c. of ammonia water are then added and the flask is further shaken at intervals during half an hour. Now 10 c.c. of distilled water are added and the liquid is shaken until the powder collects in compact masses, so that 100 c.c. of the clear ethereal solution (= 10 grm. of the root) can be poured off into a measuring cylinder. The 100 c.c. of solution is then transferred to a separator and shaken, first with 10 c.c. N sulphuric acid + 10 c.c. water, then with 3 c.c. N sulphuric acid + 5 c.c. water and finally with 10 c.c. of water. The aqueous extracts, after mixing, are rendered alkaline with ammonia and shaken successively with 25, 20 and 10 c.c. of ether. The three ethereal extracts are evaporated together and the residue is dissolved in 12 c.c. $N/10$ sulphuric acid, by gently warming, if necessary. After adding 5 drops of cochineal solution the excess of acid is titrated with $N/50$ potassium hydroxide. The number of

c.c. of $N/10$ H$_2$SO$_4$ neutralised by the alkaloid multiplied by 0.238 gives the percentage of alkaloid in the root (1 c.c. $N/10$ sulphuric acid = 0.0238 grm. alkaloid). The United States Pharmacopœia requires a minimum alkaloidal content of 1.75%.

The 1910 edition of the German Pharmacopœia gives the following *assay* method for ipecacuanha: 12 grm. of the finely powdered root are placed in a flask with 90 grm. ether and 30 grm. chloroform, and after shaking, 5 grm. sodium carbonate solution and 5 grm. water are added. The mixture is allowed to stand for 3 hours, with frequent shaking. 60 grm. of the solution (= 6 grm. of the drug) are filtered through a well covered filter, and the solvent is distilled off in a flask. The residue is warmed with 10 c.c. of 0.125% hydrochloric acid and this is filtered through a wet filter. The extraction is repeated twice, each time with 10 c.c. of the same acid; the filter is washed with water and the filtrate and washings, after being made alkaline with sodium carbonate, are shaken for 2 minutes with 5 c.c. of chloroform; the extraction is repeated 3 times with 5 c.c. of chloroform. To the 4 chloroform extracts, after mixing, 10 c.c. of $N/10$ hydrochloric acid is added and then enough ether to make the chloroform ether layer float on top of the aqueous layer. After shaking for 2 minutes the aqueous layer is filtered through a wet filter and the ethereal layer is washed by shaking with three successive quantities of 10 c.c. of water. The acidic and aqueous filtrates are collected in a 100 c.c. measuring flask and made up to 100 c.c.; 50 c.c. (= 3 grm. of the drug) are removed and added to 50 c.c. of water, together with a freshly prepared solution of a particle of hæmatoxylin in 1 c.c. of alcohol. The mixture is titrated with $N/10$ potassium hydroxide, until a strong yellow colour is produced, changing rapidly to bluish-violet on shaking. At most 2.6 c.c. of $N/10$ potassium hydroxide should be required, so that at least 2.4 c.c. of $N/10$ acid was neutralised by the alkaloids, *i.e.*, the alkaloidal content of the root should be at least 1.99%, calculated as emetine.

It is probable that in the last-mentioned process, cochineal would be a better indicator than hæmatoxylin.

The proportion of alkaloids present in ipecacuanha-root is subject to much variation, and owing to the variety of methods employed the published results show still wider discrepancies.

The following table, expanded from one by R. A. Cripps (*Pharm. J.*, 1895, [iii], **25**, 1093), shows the proportions of mixed alkaloids

Observer	Reference	Variety of drug	No. of samples examined	Method employed	Alkaloids found, %
Lyons	Pharm. J., 1886, [iii], 16, 627	Brazilian	48	Dragendorff's (titration by Mayer's solution).	Near 2.60 (5 under 2.0 p.c.) (10 over 3.0 p.c.)
Hooper	Pharm. J., 1891, [iii], 22, 591	E. Indian root	1	Extraction by alcohol and titration by Mayer.	1.79
Hooper	Pharm. J., 1891, [iii], 22, 591	E. Indian stem	1	Extraction by alcohol and titration by Mayer.	1.13
Kottmayer	Pharm. Post, 1892, pp. 913, 935	Brazilian	Not stated	Extraction by acidified alcohol, purification by lead acetate and lime, and solution in chloroform.	2.32
Kottmayer	Pharm. Post, 1892, pp. 913, 935	E. Indian	Not stated		2.26
Kottmayer	Pharm. Post, 1892, pp. 913, 935	Carthagena	Not stated		1.81
Caesar & Loretz	Apoth. Zeit., 7, 464	Brazilian	3	Not stated	1.05
Caesar & Loretz	Pharm. J., 1892, [iii], 23, 267	E. Indian	1	Not stated	0.54
Caesar & Loretz	Pharm. J., 1892, [iii], 23, 267	Carthagena	3	Not stated	1.38
Keller	Zeit., 38, 23	Brazilian	7	Extraction by ammoniacal ether and chloroform, separation by acid, liberation by ammonia, and titration.	2.58
Keller	Zeit., 38, 23	Carthagena	3		2.18
Attfield	Pharm. J., 1893, [iii], 24, 48	Brazilian root	2	Extraction by cold ammoniacal chloroform, followed by hot ammoniacal chloroform, and separation.	2.01
Attfield	Pharm. J., 1893, [iii], 24, 48	Brazilian stem	2		1.68
Paul & Cownley	Pharm. J., 1893, [iii], 24, 62	Brazilian root	8	Not stated	2.11
Paul & Cownley	Pharm. J., 1893, [iii], 24, 62	Brazilian stem	3	Not stated	1.25
Paul & Cownley	Pharm. J., 1893, [iii], 24, 62	Carthagena	Not stated	Not stated	About 2.0
Arndt	Apoth. Zeit., 1890, p. 781		12	Modified Flückiger (hot ammoniacal chloroform).	0.6 to 1.1
Cripps & Whitby	Pharm. J., 1889, [iii], 19, 724		12	Ethyl acetate extraction.	1.21
R. A. Cripps	Pharm. J., 1895, [iii], 25, 1094	Brazilian	61	By ethyl acetate or Lyons' process modified.	1.97
R. A. Cripps	Pharm. J., 1895, [iii], 25, 1094	Brazilian stem	1		2.24
R. A. Cripps	Pharm. J., 1895, [iii], 25, 1094	Carthagena	5		1.70
Frerichs & de Fuentes Tapis	Arch. Pharm., 1902, 240, 390	Brazilian		Extraction by ammoniacal ether, separation by acid, liberation by ammonia and weighing or titration.	1.81
Beckurts	Apoth. Zeit., 1903, 18, 102	Brazilian		As above.	2.4 to 3.3
Beckurts	Apoth. Zeit., 1903, 18, 102	Carthagena		As above.	2.006 to 2.397
Carr and Reynolds	Pharm. J., 1908, [iv], 26, 542	Brazilian		Not stated	1.903 to 2.387
Carr and Reynolds	Pharm. J., 1908, [iv], 26, 542	Brazilian		Not stated	1.76 to 2.77, 0.98 to 1.83 emetine, 0.48 to 1.29 cephaëline

found by various observers in commercial ipecacuanha, together with the outline of the methods employed for its estimation.

De-emetinised Ipecacuanha has been found by Kanthack and Caddy (*Practitioner*, June, 1893, page 411; *Pharm. J.*, 1893, [iii], **23**, 990) to be of great value in the treatment of dysentery. They found the anti-dysenteric value to be in direct proportion to the amount of alcohol-soluble substances present, provided that the emetine had been completely removed, or existed only in minute quantity. As a fact, the proportion of emetine in the so-called de-emetinised root ranged from traces to 1.2%, while the yield of alcoholic extract was from 2.5 to 11.3%. The sample which gave the best results was prepared by Merck, and showed on analysis only traces of emetine, with 10.3% of alcoholic extractive. B. H. Paul found nearly 0.5% of emetine in de-emetinised ipecacuanha from the same source (*Pharm. J.*, 1893, [iii], **24**, 212). The nature of the antidysenteric principle in ipecacuanha has not been definitely ascertained.

The Ipecacuanhas of English Commerce form the subject of an interesting paper by E. J. Holmes (*Pharm. Jour.*, 1893, [iii], **24**, 209. See also H. G. Greenish, *ibid.*, pp. 383, 391; 1894, [iii], **25**, 689; J. Moeller, 1893, [iii], **24**, 209, and C. Hartwich, p. 1088). Spurious ipecacuanhas have been described by T. H. Wardleworth (*Pharm. J.*, 1892, [iii], **23**, 250), R. A. Cripps (*ibid.*, 1893, [iii], **24**, 399), Ranwez and Campion (*Ann. de Pharm.*, **1**, 238), and others.

Alkaloids of Pomegranate.

The bark of the pomegranate (*Punica granatum*) contains several alkaloids, which are liquid at the ordinary temperature (see Vol. 6, p. 230).

Pelletierine, $C_8H_{15}ON$, is a liquid of a peculiar odour, and a sp. gr. of 0.988 at 0°. It boils and distils at 195°, and darkens and resinifies on exposure to air. Pelletierine is dextrorotatory, soluble in 20 parts of cold water, and readily soluble in alcohol, ether, and chloroform. The alkaloid is removed by chloroform from alkaline solutions, but not from acid or bicarbonate aqueous solutions. The solutions have an alkaline reaction. Pelletierine forms a series of crystallisable salts.

Pelletierine sulphate forms minute, white, acicular crystals, which are freely soluble in water. It is employed in medicine, its chief application being in doses of from 5 to 8 grains as a remedy for tape-worm.

The *pelletierine tannate* of commerce is a mixture of the tannates of the total alkaloids from pomegranate bark. It forms an amorphous yellowish-gray powder of astringent taste, almost insoluble in water, but soluble in alcohol and in dilute acids.

Pelletierine is precipitated from the solutions of its salts by alkali hydroxides, but not by alkali hydrogen carbonates. It is not precipitated by plantinic chloride, but gives precipitates with most other general reagents for alkaloids.

Pelletierine is associated in pomegranate bark with the closely analogous liquid alkaloids, *isopelletierine*, $C_8H_{15}ON$, and *methylpelletierine*, $C_9H_{17}ON$; and with the solid alkaloid *pseudopelletierine*, $C_9H_{15}ON$, forming crystals melting at 48°. (See Tanret, *Bull. Soc. Chim.*, 1879, **32**, 464; *Compt. Rend.*, 1880, **90**, 695.)

Assay of Pomegranate Bark.—The bark of the root is richer in alkaloids than that of the aerial portions and contains on an average 0.3 to 0.6% (Kunz-Krause). The following process for the estimation of the alkaloids has been suggested by Gehe & Co. (*Handelsbericht*, Sept., 1893, 37). 20 grm. of the coarsely powdered root are macerated for half a day with a mixture of 40 grm. chloroform, 60 grm. ether and 20 grm. 10% ammonia. The mixture is shaken from time to time, and finally 50 grm. of the ether-chloroform solution (= 10 grm. of the bark) is filtered off and concentrated to one-fifth of its volume. After the addition of 10 c.c. of $N/10$ sulphuric acid the rest of the chloroform and ether is evaporated. The acid solution is then filtered and the flask rinsed out with 2 or 3 c.c. of water, which are used to wash out the filter. In the filtrate the excess of acid is titrated back with $N/10$ sodium hydroxide, using cochineal tincture as indicator. The number of c.c. of acid used up, multiplied with 0.1475 (from average of the equivalents of the four alkaloids) gives the percentage total alkaloid of the bark. Two specimens of Javanese bark were found by this process to contain 1.15 and 1.24% of alkaloids. Carr and Reynolds (*Pharm. J.*, 1908, [iv], **26**, 542) found in a large number of commercial specimens 0.12 to 0.29% of total alkaloid; their method of assay is not given.

The Alkaloids of Jaborandi.

Pilocarpine was first isolated from the leaves of the true Jaborandi (*Pilocarpus jaborandi*) which contain in addition the isomeric alkaloid *iso*pilocarpine and a third alkaloid, pilocarpidine. Pilocarpine and

*iso*pilocarpine are also found in other species of *Pilocarpus* (*P. microphyllus*, *P. pennatifolius*), but pilocarpidine has only been found in *P. jaborandi*.

For the preparation of the alkaloid, the leaves of *Pilocarpus* are extracted with rectified spirit to which 1% of strong ammonia has been added. After neutralisation with tartaric acid, the tincture is evaporated, and the residue, after making alkaline with ammonia, is extracted with chloroform. The impure pilocarpine left on evaporation of the chloroform, is converted into the nitrate, or the chloroform solution is at once shaken out with dilute nitric acid. The nitrate, which separates out, is crystallised from boiling alcohol. For the preparation of tincture of jaborandi see Farr & Wright (*Pharm. J.*, 1891, [iii], **21**, 1).

Pilocarpine, $C_{11}H_{16}O_2N_2$, the principal alkaloid, was discovered by Hardy (*Bull. Soc. Chim.*, 1875, [ii], **24**, 497) and independently by Gerrard (*Pharm. J.*, 1875, [iii], **5**, 865, 965; **7**, 225).

The first complete account of pilocarpine and its salts was given by Petit and Polonowsky (*J. Pharm. Chim.*, 1897, [vi], **5**, 370, 430, 475; **6**, 8) and their description has, in the main, been confirmed by Jowett (*Trans.*, 1900, **77**, 473) from whose papers the following data are taken.

The free base is best prepared by adding excess of ammonia to an aqueous solution of one of its salts, extracting with chloroform, and evaporating. It forms a colourless thick oil, which has been crystallised with difficulty, forming long needles, m. p. 34° (Pinner & Schwarz, *Ber.*, 1902, **35**, 210); it is freely soluble in water, alcohol and chloroform, but almost insoluble in ether and light petroleum. On distillation in a vacuum it is partially changed to *iso*pilocarpine. The base is a lactone and has acidic properties, so that it cannot readily be extracted from a solution made strongly alkaline with sodium hydroxide (Petit and Polonowsky). Pilocarpine is dextrorotatory; in chloroform solution $[\alpha]_D = +100.5°$.

The most characteristic salt is the nitrate, $C_{11}H_{16}O_2N_2$, HNO_3, m. p. 178° (corr.) dissolving at 20° in 6.4 parts of water and in 269.3 parts of absolute alcohol. The *hydrochloride*, $C_{11}H_{16}O_2N_2$,HCl, forms large crystals from strong alcohol, m. p. 204 to 205°; the *hydrobromide* melts at 185° (corr.). The *sulphate*, m. p. 132° (corr.) is very soluble in alcohol and in water; the *picrate*, m. p. 147°, the *aurichloride*, $C_{11}H_{16}O_2N_2$,HAuCl$_4$,H$_2$O, m. p. 117 to 130°, the *platinichloride*, $(C_{11}H_{16}O_2N_2)_2$,H$_2$PtCl$_6$, m. p. 218° (corr.) and a *gold double salt*, $C_{11}H_{16}O_2N_2$,AuCl$_3$, m. p. 163° (corr.) have also been prepared.

Isopilocarpine is the name suggested by Jowett for an isomeride, readily formed from pilocarpine by alkalies or by distillation, which was described by Petit and Polonowsky as pilocarpidine and was by them erroneously supposed to be identical with the pilocarpidine of Harnack and Meyer isolated from *P. Jaborandi.* The free base is very similar to pilocarpine, b. p. 261° at 10 mm.; $[\alpha]_D +42.8°$. According to Petit and Polonowsky it forms large, hygroscopic crystals, but this statement has not been confirmed.

Isopilocarpine Nitrate, $C_{11}H_{16}O_2N_2,HNO_3$, melts at 159° (corr.) and is soluble in 8.4 parts of water at 19° and in 357.3 parts of absolute alcohol at 20°. The *hydrochloride* is very soluble, but not hygroscopic like pilocarpine hydrochloride. When air-dry it has the composition $(C_{11}H_{16}O_2N_2,HCl)_2,H_2O$ m. p. 127° (corr.); the anhydrous salt melts at 159° (corr.).

Pilocarpidine, $C_{10}H_{14}O_2N_2$, was first obtained by Harnack and Meyer and later by Merck from the mother-liquor of the pilocarpine nitrate from jaborandi leaves. Jowett failed to obtain it from the pilocarpine nitrate of commerce, but he found that it occurred in the leaves of the true jaborandi (*P. jaborandi*), but not in *P. micropyhllus* or *P. pennatifolius*. The true Jaborandi is now almost unobtainable and *P. microphyllus* is used in its place.

Pilocarpidine nitrate, $C_{10}H_{14}O_2N_2,HNO_3$, melts at 137° (corr.) and has $[\alpha]_D = + 73.2°$; at 15° it dissolves in 2 parts of water and 81 parts of absolute alcohol. The *aurichloride* melts at 124 to 125°; (corr.) the *platinichloride*, $(C_{10}H_{14}O_2N_2)_2,H_2Pt\ Cl_6,4H_2O$, melts at 88 to 89° (corr.), but when anhydrous, at 187° (corr.). Unlike pilocarpine and *iso*pilocarpine, pilocarpidine does not readily yield a crystalline picrate.

Jaborine of Harnack and Meyer (*Annalen*, 1880, **204**, 82) is according to Jowett a mixture of *iso*pilocarpine and pilocarpidine, possibly also containing pilocarpine.

The constitution of pilocarpine has been established by the work of Jowett, and of Pinner and his collaborators. The formula

$$C_2H_5 . \overset{*}{C}H . CH . CH_2 . C . N(CH_3)$$

CO   CH₂   CH—N CH

O
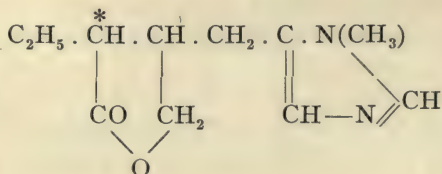
was suggested for *iso*pilocarpine by Pinner and Schwarz (*Ber.*, 1902, **35**, 2441) and has been confirmed by Jowett. According to Pinner (*Ber.*, 1905, **38**, 1510) pilocarpine is structurally isomeric with *iso*pilocarpine, but Jowett (*Trans.*, 1905, **87**, 794) regards the two bases as stereo-isomeric. He was able partially to convert pure *iso*pilocarpine into pilocarpine by heating with alkali, so that the effect of alkali on either alkaloid is to produce an equilibrium mixture (consisting mostly of *iso*pilocarpine). According to Jowett the interconversion is due to the racemisation of that one of the two asymmetric carbon atoms which is remote from the glyoxaline ring, and to which the ethyl group is attached (marked by * in the above formula). The constitution of *iso*pilocarpine is principally based on the formation of two lactone acids, $C_7H_{10}O_4$ and $C_8H_{12}O_5$ (*pilopic* and *homopilopic* acid), by oxidation of *iso*pilocarpine with potassium permanganate and on the subsequent oxidation of homopilopic acid to α-ethyltricarballylic acid, $C_8H_{12}O_6$, and further on the production of methyl-, dimethyl- and methyl-amyl-glyoxaline on distillation with soda lime. The complete proof of the identity of the glyoxaline derived from *iso*pilocarpine has lately been furnished by Pyman (*Trans.*, 1910, **97**, 1814).

For the *estimation* of pilocarpine (mixed with *iso*pilocarpine), Fromme (Caesar and Loretz's *Geschäftsbericht*, 1901, **27**; *Apoth. Zeit.*, 1902, **17**, 183) recommends the following process: 15 grm. finely powdered Jaborandi leaves are macerated with 150 grm., chloroform and 15 grm. ammonia water for half an hour; the mixture is frequently shaken. More than 100 grm. of the chloroform solution is then collected and is shaken after the addition of 1 grm. water, so that, on standing, the leaf particles at first remaining in suspension, settle down and the chloroform solution becomes quite clear. After one hour 100 grms. of the clear solution is weighed out, and shaken successively with 30, 20 and 10 c.c. of dilute hydrochloric acid. The mixed acid solutions are then washed with 20 c.c. of ether, rendered alkaline with ammonia and extracted with 30, 20 and 10 c.c. of chloroform. The chloroform on evaporation leaves behind the alkaloid from 10 grm. of leaves.

Matthes and Rammstedt (*Arch. Pharm.*, 1907, **245**, 112) concentrate the final chloroform solution to 20 c.c. and add 3 c.c. of 0.1 N picrolonic acid in alcohol; then 60 c.c. of ether are added to precipitate the picrolonate which is collected after 24 hours, filtered, washed with a mixture of 0.5 c.c. alcohol with 1.5 c.c. ether, dried and weighed.

In the British Pharmacopœia (1898) *P. jaborandi* is the only official species: the United States Pharmacopœia (1905) admits also *P. microphyllus* and requires a minimum alkaloidal content of 0.5% of alkaloid, as estimated by the following process: 10 grm. of the leaves, in No. 60 powder, are moistened with 2 c.c. of ammonia water and 3 c.c. of chloroform and are at once packed in a small percolator. They are percolated with chloroform containing 2% of ammonia water until 100 c.c. have been collected. The percolate is then shaken successively with 15 c.c. *N* sulphuric acid, 2 c.c. *N* sulphuric acid mixed with 8 c.c. of water, and finally with 10 c.c. of water. These three extracts are mixed, made alkaline with ammonia and shaken successively with 20, 15 and 10 c.c. chloroform. The three chloroform extracts are mixed, and evaporated on the water-bath; the residue is dissolved in 7 c.c. of $N/10$ sulphuric acid and the excess of acid is titrated with $N/50$ potassium hydroxide, using 5 drops of cochineal or iodeosin solution as indicator. 5 c.c. of the potassium hydroxide $= 1$ c.c. of $N/10$ sulphuric acid $= 0.02$ grm. of alkaloid.

Carr and Reynolds (*Pharm. J.*, 1908, [iv], **26**, 542) found the amount of pilocarpine in commercial samples of leaves extremely variable. For *P. jaborandi* the amount of pilocarpine nitrate obtained varied from traces too small to estimate, to 0.05%; in *P. microphyllus* it varied from similar traces up to 0.99%.

Alkaloids of Pepper.

Piperine. $C_{17}H_{19}O_3N$; or $C_5H_{10}N.CO.C_4H_4.C_6H_3\langle{}^O_O\rangle CH_2$.

Piperine is an alkaloid existing in various plants belonging to the *Piperaceæ*, and is the characteristic principle of both black and long pepper.[1] It also exists in the berries of *Schinus mollis*, a tree belonging to the terebinthaceous order, and has been obtained synthetically by the reaction of its decomposition-products piperidine and piperic acid (chloride).

For the preparation of piperine, white pepper should be exhausted with rectified spirit, the tincture concentrated to an extract, and the extract mixed with sodium or potassium hydroxide solution. This dissolves resin, and leaves impure piperine, which is purified by re-

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[1] According to Dunstan and Carr *Piper ovatum* contains piperovatine, $C_{16}H_{21}O_2N$ (*Trans.* 1895, **67**, 94; *Proc. ibid.*, Nov. 7, 1895).

peated crystallisation from boiling alcohol. Winckler precipitates the alcoholic extract of pepper with basic lead acetate, exactly precipitates the lead from the filtrate by sulphuric acid (or hydrogen sulphide), filters hot, evaporates, treats the residue with water, and boils the undissolved portion with alcohol, from which piperine crystallises on cooling. Another plan is to mix powdered pepper into a paste with slaked lime and water, dry the mixture at 100°, exhaust it with boiling ether, and crystallise from alcohol the piperine left on evaporating the ether. By this process, Cazeneuve and Caillot found in Sumatra pepper an average proportion of 8.10% of piperine; in white Singapore pepper, 7.15; and in black Singapore pepper, 9.15%.

Piperine forms pale lemon-yellow, nearly tasteless, four-sided, monoclinic prisms. It melts at 131° (uncorr.), but at a lower temperature if impure. Above its melting-point it turns brown, and undergoes decomposition.

Piperine is insoluble in cold water, and very slightly soluble in boiling water. It dissolves readily in alcohol, the solution being optically inactive, and destitute of alkaline reaction, but having an extremely pungent taste, like that of pepper. Piperine dissolves sparingly in ether, but with facility in chloroform, benzene, and petroleum spirit.

Piperine is a feeble base. Its salts are decomposed by excess of water with precipitation of free piperine, and the alkaloid is extracted even from its acidified solutions by agitation with chloroform, benzene, petroleum spirit, etc. *Piperine hydrochloride* is soluble in alcohol, and on treating the solution with alcoholic mercuric chloride or platinic chloride the corresponding compounds are obtained as crystalline precipitates.[1]

On adding iodised potassium iodide to a hot alcoholic solution of piperine, acidified with hydrochloric acid, an iodo-compound is formed which separates on cooling in fine steel-blue needles.

Concentrated nitric acid converts piperine into an orange-red resinous substance which is turned blood-red by alkali hydroxide with formation of piperidine.

With concentrated sulphuric acid piperine instantly gives a blood-red colouration, becoming brown on warming or standing. On addition of water, piperine is precipitated.

[1] The composition of these salts is said to be respectively $(C_{17}H_{19}O_3N)_2$, HCl, HgCl_2, and $(C_{17}H_{19}O_3N)_4$, 2HCl, PtCl_4.

When boiled with strong alkali hydroxide, or heated with soda-lime, piperine undergoes hydrolysis with formation of piperic acid and piperidine, thus:

$$C_{17}H_{19}O_3N + H_2O = C_{10}H_{12}O_4 + C_5H_{11}N.$$
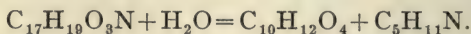
Piperidine is hexahydropyridine, and has already been described (Vol. 6). Traces (0.01%) of a volatile base occur in pepper. According to Pictet and Court (*Ber.*, 1907, **40**, 3776) this base is not piperidine, but probably methyl pyrroline, C_5H_9N.

Piperic Acid, $C_{12}H_{10}O_4$; or $CH_2:O_2:C_6H_3.CH:CH.CH:CH.COOH.$ Piperic acid is the acid product of the hydrolysis of piperine. It is best prepared by boiling a solution of equal weights of piperine and potassium hydroxide in a minimum quantity of strong alcohol for 5 or 6 hours under a reflux condenser. The crystalline plates of potassium piperate, which are separated from the brown mother-liquor, recrystallised from boiling water, redissolved in boiling water, the solution decolorised by animal charcoal, and treated with hydrochloric acid, when the piperic acid which separates as a jelly, is washed and recrystallised from alcohol.

Piperic acid crystallises from alcohol in long yellowish needles, often felted together, but when precipitated by adding an acid to the solution of one of its salts it usually separates as a sulphur-yellow jelly, which shrinks on drying. Piperic acid melts at 216 to 217⁹, and at a higher temperature partially sublimes with an odour of coumarin, leaving a brown residue. It is nearly insoluble in water, and requires 270 parts of cold alcohol for solution, but dissolves readily in boiling alcohol. It is sparingly soluble in ether and benzene, and nearly insoluble in petroleum spirit.

Piperic acid is a feeble acid, but forms a series of crystallisable salts, most of which are but sparingly soluble in water, and insoluble in alcohol.

Concentrated sulphuric acid turns piperic acid blood-red, and subsequently chars it. Nitric acid, even when dilute, converts it into a nitro-derivative of an orange colour, which evolves an odour of coumarin when heated with alkali hydroxide. When fused with potassium hydroxide, piperic acid yields protocatechuic, acetic, oxalic, and carbonic acids, with hydrogen and secondary products.

Commercial Pepper.—The pepper of commerce, when genuine, consists of the immature dried fruit of *Piper nigrum*, a plant indigenous

to India, and at present cultivated in the West Indies, various parts of
the Malayan Archipelago, and other tropical districts.[1]

Black pepper is composed of the entire berries, with the pulp
adhering, gathered before they are quite ripe, and dried in the sun.
White pepper consists of the decorticated berries, and hence shows a
much smaller proportion of ligneous matter on analysis. To meet the
demand for a very light-coloured pepper, the outer layers of the seed
are sometimes ground off, and the nearly white kernel alone used.
Such pepper contains a large proportion of starch, but is deficient in
flavour and pungency.

The commercial value of pepper depends much upon the weight
of the peppercorns. The following figures show the weight in grm. of
100 berries of the chief commercial varieties of pepper as observed by
A. Wynter Blyth and W. Johnstone.

	A. W. Blyth	W. Johnstone
Penang	6.2496	3.9028
Penang, white		4.9360
Malabar	6.0536	
Sumatra	5.1476	
Trang	4.5736	4.8101
Tellicherry	4.5076	4.4421
Acheen		5.1976
Alleppy		3.8438
Kampoot		4.4540
Lampong		3.5410
Siam		4.2776
Siam, white		5.1441
Singapore		4.5338
Singapore, white		4.6936

Blyth remarks that, in the trade, Malabar pepper is generally con-
sidered the heaviest, and that his sample of Penang may have been
particularly fine. As retailed in a ground state, commercial pepper

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[1] **Cayenne pepper** is quite distinct in composition, characters, and botanical origin from
true pepper. It consists of the ground pods of *Capsicum frutescens* or *C. fastigatum*.
The following figures are the average of several analyses of commercial cayenne pepper
by A. Wynter Blyth:

     Aqueous extract..32.10%
     Alcoholic extract..25.79%
     Benzene extract..20.00%
     Ethereal extract...10.73%
     Total nitrogen.. 2.04%
     Ash.. 5.69%

The pungency of cayenne pepper is due entirely to the oil and resin extractable by ether.
After exhaustion with this solvent the residue is tasteless, or nearly so.
When added to mustard, gin, etc., cayenne pepper can be detected by the pungent taste
of the alcoholic or ethereal extract. A highly characteristic behaviour is the production of
intensely irritating vapours on heating the residue. On inhaling these cautiously, the
burning sensation produced in the throat and lungs is not to be mistaken.
The larger entire, dry, berry-like fruits of *Capsicum annuum*, *C. longum*, etc., are known
as *Paprika* or Spanish pepper (Spaeth, *Zeit. Nahr. Genussm.*, 1905, **10**, 27).

is invariably blended, a common mixture consisting of equal parts of
Malabar, Penang, and Sumatra pepper. Of these, the first, which
is the dearest gives weight, the second strength, and the third colour.

F. Härtel and R. Will (*Zeit. Nahr. Genussm.*, 1907, **14,** 574) found
the weight of 100 peppercorns for 15 varieties of black pepper to vary
between 2.14 and 4.85 grm. G. Graff (*Zeit. oeffentl. Chem.*, 1908, **24,**
425) examined 12 samples of black pepper; the weight of 50 c.c.
varied from 13.05 to 26.97 grm. and the number of peppercorns in
this quantity from 520 to 1705.

The constituents peculiar to pepper, which to some extent determine
its value as a spice, are a *resin*, a *volatile oil* and the alkaloid *piperine*,
but it is impossible to base an estimate of the value of pepper on the
amounts of these substances present. Indirect means must be em-
ployed in detecting adulteration of ground pepper, which "is perhaps
the most extensively adulterated of all food products, bran, hulls and
other by-products from wheat, maize, rice, oats, buckwheat and other
grains, ground linseed, olive and rape seed cake, shells (often roasted
or charred) of cocoanut, almonds and other nuts, sawdust, mustard,
husks of cayenne pepper, long pepper, pepper shells, terra alba, etc.,"
being employed for this purpose. (Winton, *Connecticut Exper.
Stat. Report*, 1896, **20,** 32.) Of 102 specimens of ground pepper bought
retail in America and examined by Winton, 64 were pure, 12 suspect,
31 certainly adulterated. More recent writers in Germany and
Kraemer and Sindall in America (*Amer. J. Pharm.*, 1908, **80,** 1) have,
however, expressed the opinion that as a result of more rigorous chem-
ical control, the grosser forms of adulteration are now disappearing.

There is, however, a practice which, if it is to be considered an adul-
teration at all, is of too subtle a nature to allow of invariable detection,
namely, the addition to black pepper of a certain amount of "pepper
shells," the by-product obtained in the manufacture of white pepper.
Although the shells (*i.e.*, the dark pericarp of the berry) contain a far
higher proportion of crude fibre than white pepper, and therefore also
a higher proportion than black pepper (=white pepper + shells) it is
possible, according to Graff, by adding 30% of shells to a good black
pepper with little crude fibre, to obtain a mixture of middling quality,
which still has a percentage of crude fibre within normal limits, and
which cannot of course be distinguished from pure black pepper by
microscopical examination. Graff states that such black pepper mixed
with shells is largely imported from England to Germany, and he

would consider this practice permissible, but Spaeth and Winton regard it as an adulteration.

Added constituents, derived from other plants, are best detected under the microscope. For the microscopical appearance of normal pepper and its adulterants the above-mentioned paper by Kraemer and Sindall may be consulted; also, A. L. Winton, and J. Moeller, *The Microscopy of Vegetable Foods*, New York, 1906 (John Wiley and Sons), pp. 502–525, which contains an extensive bibliography of the subject; A. E. Leach, *Food Inspection and Analysis*, 2nd edition, New York, 1911 (John Wiley and Sons), pp. 428–445 and plates xxxiii–xxxvii; H. G. Greenish, *The Microscopical Examination of Foods and Drugs*, 2nd edition, London, 1910 (J. and A. Churchill), pp. 279–287; E. J. Parry, *Food and Drugs*, London, 1912, (Scott Greenwood); Villiers and Collin, *Traité des Altérations et falsifications des substances alimentaires*, Paris, 1900. (Compare also Collin, *Ann. des Falsifications*, 1910, **3, 272.**)

The addition of foreign substances is further revealed by chemical analysis, which alone is available for the detection of an undue proportion of pepper shells in ground black pepper. The interpretation of the analyses in the latter case is based on the fact that the shells contain less non-volatile ether extract, less piperine, less starch, but more ash and more crude fibre than white pepper, and consequently also than pure black pepper. In the "Standards of purity for food products" (*U. S. Department of Agriculture, Bureau of Chemistry, Bull.* No. 69 (revised), Part 1, 1905, p. 18) standard black pepper is defined as black pepper free from added pepper shells, pepper dust and other pepper by-products, and containing not less than 6% non-volatile ether extract, not less than 25% starch, not more than 7% ash, of which not more than 2% is insoluble in hydrochloric acid (sand), and not more than 15% crude fibre. The non-volatile ether extract should contain at least 3.25% nitrogen. These official figures are principally based on Winton's results. Spaeth (*Zeit. Nahr. Genussm.*, 1905, **10, 30**) suggests for air-dry black pepper the same lower limit for the non-volatile ether extract and for its nitrogen content, and the same upper limits for the total ash and for the ash insoluble in hydrochloric acid. For crude fibre he gives the more liberal upper limit of 17.5%. (Härtel and Will give 17%, Hass and Hoernstein, *Zeit. Nahr. Genussm.*, 1910, **20,** 506, suggest 16%.)

Standard *white* pepper, according to the same official American

description, should yield at least 6% of non-volatile ether extract, of which at least 1/25, or 4% of the extract, should be nitrogen. The starch should be not less than 50%, the crude fibre not more than 5%, the ash not more than 4%, and the ash insoluble in hydrochloric acid not more than 0.5%. For white pepper Spaeth suggests the same limits as the above for total ash, and non-volatile ether extract. The nitrogen content of the latter should be at least 3.5%; the ash insoluble in acid should not exceed 1%, and the crude fibre should not exceed 7%. Spaeth further states that the "lead number" according to Busse, for black pepper, should be less than 0.08 grm. of lead per grm. of water-free powder, and for white pepper less than 0.03 grm. The starch by the diastase method for black pepper is 30 to 38%, for white pepper 45 to 60%; the amount of piperine is 4 to 7.5% and 5.5 to 9.0% respectively.

Recently Hass and Hoernstein (*loc. cit.*) suggest 6.5% as the upper limit for crude fibre in white pepper.

Analytical Methods.

Crude Fibre.—Among the above limits, that of the *crude fibre* is the most important for the detection of added pepper shells and of many foreign vegetable substances. Winton, Ogden and Mitchell (*Connecticut Exp. Stat. Rep.*, 1898, **22**, 184) use the method of the A. O. A. C. (see *Bulletin* No. 107, *Bureau of Chemistry;* also Vol. 1, p. 70); that described by Spaeth, as follows, is quite similar:

3 grm. of the finely powdered pepper, which has passed a sieve with 1/2 mm. holes,[1] is extracted in a conical flask with 50 c.c. of alcohol and 25 c.c. of ether for 1 hour under a reflex condenser on the water-bath. After cooling, the alcohol-ether mixture is poured off through a Gooch crucible and the powder in the flask is washed with alcohol-ether by decantation. It is then washed into a porcelain basin, together with the asbestos from the filter and the particles retained by the latter, and is boiled for half an hour with 200 c.c. of 1.25% sulphuric acid. After settling, the acid is poured off through a Gooch crucible, the residue in the flask is boiled with 200 c.c. of water for 15 minutes, and is then washed into the crucible. The substance + asbestos is then boiled with 1.25% potassium hydroxide and boiled with water after decantation, exactly as in the case of the acid. It is collected in the crucible,

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[1] Winton, Ogden and Mitchell use a steel mill and sieve with circular holes 1/25 inch in diameter.

washed with 75 c.c. of alcohol, then with ether, and transferred with the asbestos to a platinum dish (the last traces being wiped out by means of clean asbestos). After drying for 1 hour at 105 to 110° the dish is weighed, ignited and weighed again. The difference between the two weighings represents the crude fibre.

A rapid volumetric method, for rough comparison, is also given by Spaeth; 0.5 grm. of the pepper powder is boiled with 30 c.c. of 1.5% potassium hydroxide until the starch has dissolved. After the crude fibre has settled the potash is decanted, and the fibre is washed with hot water. It is then poured into a narrow cylinder and its volume, after settling, is compared with that obtained by treating pepper with a known percentage of crude fibre by the same process.

Starch.—The estimation of the *starch* is of less value than that of the crude fibre; Graff does not consider it a suitable criterion. Winton, Ogden and Mitchell estimate the starch by *direct inversion* as follows: 4 grm. of the pepper powder is extracted with ether, washed on to a filter with 150 c.c. of 10% alcohol and the residue is then heated with 200 c.c. of water and 20 c.c. of hydrochloric acid (sp. gr. = 1.125) for 3 hours on the water-bath. The solution is nearly neutralised and made up to 500 c.c. The dextrose is then determined by Allihn's method. Spaeth, in applying the *diastase* method, boils 5 grm. with 200 c.c. distilled water for 2 hours under a reflux condenser, cools to 65°, adds 0.1 grm. diastase and keeps the solution at this temperature for 4 to 5 hours. He then adds 25 c.c. of lead acetate solution, makes up to 250 c.c., allows to stand for 1 hour, with frequent shaking, filters off 200 c.c., precipitates the excess of lead in this filtrate by adding 25 c.c. of saturated sodium phosphate solution, nearly neutralises with acetic acid, adds 20 c.c. of 25% hydrochloric acid, heats for 2 hours under reflux condenser in a boiling water-bath, neutralises with the calculated amount of potassium hydroxide and then determines the sugar with Fehling's solution (see Vol. 1).

Non-volatile ether extract is, after the crude fibre determination, one of the best criteria for detection of adulterations. Winton, Ogden and Mitchell extract 2 grm. with absolute ether for 20 hours, leave the extract for 18 hours over sulphuric acid, dry, weigh (to find total ether extract), dry at 100° for 6 hours, and then at 110° until constant.

Nitrogen in the non-volatile ether extract is almost wholly present as *piperine*, and probably affords a better method of estimating

this alkaloid than the direct methods which have been suggested (*e.g.*, by Hilger and Bauer). As piperidine is formed, it is, however, essential to use the Gunning-Arnold modification of the Kjeldahl method. Härtel and Will (*Zeit. Nahr. Genussm.*, 1907, **14**, 567; Abstract, *Analyst*, 1908, **33**, 18) extract 10 grm. of pepper for 4 hours with ether and heat the extract so obtained with 1 grm. yellow mercuric oxide, 1 grm. copper sulphate, 20 grm. potassium sulphate and 25 c.c. sulphuric acid, until all has dissolved and the original colour has become a pure green. After cooling the contents of flask is dissolved in water and distilled with 150 c.c. of 30% sodium hydroxide, 100 c.c. of 5% potassium sulphide and 2 grm. of talcum. The ammonia is collected in 50 c.c. of $N/10$ sulphuric acid and titrated with Congo Red. The number of c.c. of $N/10$ sulphuric acid neutralised multiplied by 0.0285 gives the amount of piperine present.

Ash is estimated by incineration of 2 grm. and the portion of this *ash insoluble in acid* by boiling it with 25 c.c. of 10% hydrochloric acid and collecting the residue in a Gooch crucible (Winton, Ogden and Mitchell). In genuine pepper this residue mostly consists of sand, originally adhering to the peppercorns.

The above methods of analysis have all been largely used in most of the recent analyses of pepper. A few others, which have not met with general acceptance may be mentioned.

W. Busse (abst. *Analyst*, 1895, **20**, 180) has devised a peculiar and somewhat troublesome process for detecting the addition of shells to black pepper. · According to Spaeth, some care is needed to ensure accuracy; when properly applied, the process would appear to give results ranking with those of the crude fibre determination. Busse determines the brown colouring substances peculiar to the husk and for this purpose treats 5 grm. of the finely powdered and dried pepper with boiling absolute alcohol, grinds up the dried residue with a little water, washes it into a flask with from 50 to 60 c.c. of boiling water, and adds 25 c.c. of a 10% solution of sodium hydroxide. The flask is warmed on the water-bath for five hours with frequent agitation, after which the contents are nearly neutralised with strong acetic acid, 250 c.c. of water added, and the liquid filtered (with the aid of a filter-pump) after standing at rest for twelve hours. 50% of the filtrate is next acidified with acetic acid, and 20 c.c. of a 10% solution of lead acetate in dilute acetic acid added. The liquid is then diluted with water to 100 c.c., agitated, and filtered. 10 c.c. of the filtrate

should be treated with 5 c.c. of dilute sulphuric acid (1:3) and 30 c.c. of alcohol, and the resultant lead sulphate filtered off, washed, weighed, and calculated to its equivalent of metal. The amount of lead thus found Busse calls the "lead number" of the sample. The extract from 100 parts of pepper, etc., gives the following percentage of lead when thus treated:

White pepper,	0.6 to 2.7% of lead.
Black pepper,	5.4 to 7.5% of lead.
Pepper husks,	12.9 to 15.7% of lead.
Pepper dust,	10.9 to 12.2% of lead.

Compare with this the superior limits of 0.03 grm. lead per gram of pepper = 3% and 0.08 grm. = 8%, suggested respectively for white and for black pepper by Spaeth (see above).

The estimation of the ethereal oil has been carried out by Härtel and Will (*loc. cit.*) by mixing 15 grm. powdered pepper in a flask with a little water, distilling with steam, saturating the distillate with sodium chloride and extracting it with 50 c.c. of pure pentane used in one lot. The salt solution and pentane are transferred to a flask of 1.5 liters capacity, the neck of which has 3 marks, indicating 2 volumes of 25 c.c. each. By adding salt solution the division between salt solution and pentane is made to coincide with the lowest mark, and by adding pentane the upper layer is made to extend to the highest mark. 25 c.c. of the pentane layer is removed with a pipette and evaporated at room temperature under greatly reduced pressure, by sucking over it with the filter pump a current of dry air, until the air no longer burns at the exit.

The determination of the *alcoholic extract* (*i.e.*, resin + piperine) and of the *piperine* contained in it by dissolving out the piperine from this extract by means of light petroleum (Stevenson, *Analyst*, 1887, **12,** 144; Heisch, *ibid.*, 1886, **11,** 188) appear to be of little value in judging pepper, and the same may be said of the estimation of total nitrogen as carried out by Graff.

For the detection of pepper shells their larger yield of furfural (estimated as hydrazone) as compared with white pepper, has been recommended.

Finally, mention may be made of a simple process of estimating added and adherent mineral matter as distinguished from the natural ash-constituents, by shaking 10 grm. of powdered pepper in a tapped funnel with chloroform and allowing the mineral constituents to settle, after which they can be removed through the tap.

Analytical Results.

A large number of the older analyses are given in König. Compare also Winter Blyth.

In the *Reports of the Connecticut Experimental Station* a large number of analyses, chiefly of sample obtained retail, are quoted. For the composition of various varieties of pure pepper, obtained wholesale, consult the papers by Spaeth, Härtel and Will, and Graff already referred to. Gladhill (*Amer. J. Pharm.*, 1904, **76,** 71) analysed many commercial specimens from the American wholesale market and similar results were also published by Kraemer and Sindall (*ibid.*, 1908, **80,** 71). Graff analysed black Java pepper before and after the decortication in a German spice-mill, together with the by-product. The amount of white pepper produced was 66%, that of the by-product ("pepper shells") 34% of the pepper employed.

	Mois- ture	Ash	Ash in- soluble in acid	Crude fibre	Starch	Total N	Ether sol. N calcu- lated as piperine
Entire Java pepper.........	11.99	4.42	0.23	11.67	33.715	1.64	4.42
White pepper from above...	11.46	2.90	0.215	6.69	41.915	1.79	5.56
By-product (shells).........	11.61	7.10	0.30	18.68	21.735	1.65	3.82

The figures are percentages of the air dry material. They show that pepper shells have more ash, more crude fibre, less starch and less piperine than the white pepper produced from the same sample.

The extreme variations in twelve samples of black pepper examined by Graff, and in fifteen samples of black pepper analysed by Härtel and Will are given below:

	Moisture	Ash	Ash insoluble in acid	Crude fibre	Starch "dextrose value"	Ether soluble N calculated as piperine
Graff......	9.32–11.72	3.98–6.43	0.14–1.05	10.74–22.20	11.61–32.91	3.90–9.98
Härtel and Will.	12.52–14.75	4.08–9.32	0.10–2.98	13.04–21.91	18.30–42.70	6.02–9.78

In addition Graff found the total nitrogen varied from 1.68 to 2.32% and Härtel and Will observed variations of the amount of the ethereal oil between 1.94 and 3.85%.

Kraemer and Sindall's figures for black pepper are:

	Ash	Ash insoluble in acid	Crude fibre	Starch	Volatile ether extract	Non-volatile ether extract
Lowest........	5.27	0.69	13.38	29.66	1.70	10.44
Highest........	6.91	2.08	26.10	44.24	0.50	7.78
Average.......	6.15	1.17	15.10	37.83	0.86	9.27

For white pepper W. F. K. Stock (*Analyst*, 1891, **16,** 224) found:

	Tellicherry	Siam	Lampong	Penang
Ash	1.05	1.45	2.20	2.75
Fibre	4.86	4.43	4.90	5.06

Those figures of Gladhill, which refer to white pepper, may be summarized as follows:

	Coriander	Singapore	Penang	Decorticated
Ash................	0.8 – 1.0	1.0 –1.2	2.1 –2.8	0.8 –1.9
Ether extract........	7.90–11.68	8.20–8.78	6.80–7.20	6.60–7.61
Piperine............	6.81– 9.00	6.78–7.26	5.74–6.76	6.25–7.02

Adulteration of Pepper.—A very large number of substances have been used for this purpose; only a few can be here referred to.

Long Pepper is the fruit of *Chavica Roxburghii* and does not consist merely of the berries analogous to the pepper-corns of true pepper plant.[1] As found in commerce, it is always contaminated with from 3 to 7% of insoluble sand and clay, imbedded in the crevices and irregularities of the fruit. Hence it is difficult, if not impossible, to clean long pepper before grinding, in the manner readily practised with true pepper.

As it is not possible to separate the hard husk and woody centre from the minute berries, ground long pepper contains much larger proportions of woody fibre than are characteristic of (ground) true pepper of

[1] According to J. Campbell Brown (*Analyst*, 1887, **12,** 67), long pepper bears much the same relation to true pepper that wild grass seed would bear to oatmeal. It consists of the small berries with the husks and indurated coverings hardened together and to the central woody stem, much as in pines the seed and coverings are all hardened into one cone. Long pepper is usually derived from wild plants, and is always contaminated with a quantity of dirt, picked up from the soil of the river-banks on which it grows.

the corresponding shade, though not so high a percentage of total cellulose as is contained in the most husky black pepper.   These facts are exemplified in the following figures by J. Campbell Brown, obtained by the analysis of samples of long pepper carefully cleaned by hand:

	A	B	C
Total ash..	8.91	8.98	9.61
Ash insoluble in hydrochloric acid.....................	1.2	1.1	1.5
Starch and other matter convertible into sugar.........	44.04	49.34	44.61
Albuminous matter soluble in alkali...................	15.47	17.42	15.51
Cellulose...	15.70	10.50	10.73
Alcoholic extract.....................................	7.7	7.6	10.5
Ethereal extract......................................	5.5	4.9	8.6
Total nitrogen..	2.1	2.0	2.3
×6.25 = albuminoids...........................	13.13	12.5	14.37

In these analyses it will be observed that the albuminoids, calculated in the usual manner from the total nitrogen, are sensibly less than the amount directly determined, although a notable quantity of the total nitrogen existed in a non-albuminoid form (piperine, etc.).

Long pepper contains a much smaller proportion of piperine than is present in true pepper, and the essential oil has a strong and disagreeable smell.

Long pepper is legitimately used in pickles, but in the ground state is not a recognised article of trade.   Its flavour and its smell on warming preclude its use in an unmixed state, and its unacknowledged addition to true pepper is clearly an adulteration (see *Analyst*, 1889, **14,** 107).

According to J. Campbell Brown (*Analyst*, 1887, **12,** 69) the presence of long pepper in ground pepper may be recognised by the following characters:

Any considerable proportion of long pepper imparts to the mixture its peculiar slaty *colour;* but this may be prevented to some extent by sifting out much of the darker or husky portions of the long pepper before mixing.

The *odour* of a mixture containing even a moderate proportion of long pepper is unmistakable after some experience.   The ethereal extract of the samples yields the characteristic odour of long pepper very plainly when warmed.   The *ash* of a sample containing long pepper will be excessive, especially the proportion of ash insoluble in hydrochloric acid.   This indication is particularly important when long pepper has been added to white pepper, the natural ash of which

is very small. When long pepper, from which the husk particles have been sifted out, is added to white pepper, it invariably introduces its sand with it, as also some spent bleach, if an attempt has been made to bleach it. The *woody fibre* in ground long pepper is always considerable. On spreading out a sample containing long pepper in a smooth thin layer on strong paper, by means of an ivory paper-knife, pieces of fluffy woody fibre will be detected, especially if the smooth thin layer be tapped lightly from below. These particles have a characteristic microscopic appearance. The *starch*-granules of long pepper are much larger than those of true pepper. They have a diameter of about 0.0002 inch, and hence are not much smaller than rice-starch. They appear isolated or loosely aggregated in clusters.

According to A. W. Stokes (*Analyst*, 1888, **13,** 109), any admixture of long pepper with true pepper can be detected by placing a small portion of the sample on a slip of glass, adding a drop of glycerin, covering it with thin glass, and observing it under the microscope, using a 1-inch power and the Nicol's prisms crossed so as to give a dark field. If ordinary pepper alone be present, the field will be entirely dark, but fragments of long pepper exhibit a ghostly white appearance. Stokes points out that rice exhibits a very similar appearance to long pepper when examined in this manner. The starch-granules of long pepper are well-defined and angular, but much larger than those of true pepper, approaching the size of the granules of rice-starch.

Ground *rice* is the most frequent amylaceous adulterant of commercial pepper. It can be recognised under the microscope by the size and polygonal shape of the starch-granules, and by the peculiar appearance already referred to when the sample is mounted in glycerin and examined by polarised light. The proportion of rice mixed with pepper is best inferred from the increased percentage of starch.

To facilitate the examination of pepper under the microscope, F. W. Rimmington (*Analyst*, 1888, **13,** 82) recommends that the sample should be shaken several times with alcohol and subsequently with water in a test-tube. The residue is allowed to subside, when it usually forms several strata, the uppermost of which is the most interesting. On removing a small portion of this with a pipette, and examining it with a magnifying power of about 250 diameters, every particle will be seen clearly defined, the starch-granules can be easily measured, and any foreign bodies can be recognised.

Many adulterants of pepper increase the amount of crude fibre in

the final mixture; this is in particular the case with added pepper shells (see above) and with *poivrette* or *pepperette*, the trade name of an adulterant which appears to have been widely used in France and in England. Poivrette consists of finely-ground olive-stones; it does not contain starch, but there is about 48% of crude fibre. (J. Campbell Brown, *Analyst*, 1887, **12**, 72, where the microscopic appearance is also described.) Various colour reactions for the detection of this substance are based on staining the woody cells it contains by means of such reagents as phloroglucinol in hydrochloric acid (cherry-red colouration, Martelli, Abst. *Analyst*, 1895, **20**, 181).

Alkaloid of the Common Broom.

The shoots of *Cytisus scoparius* (*Spartium scoparium*) contain the alkaloid *sparteine*, which may be isolated by extracting broom tops with water containing a little sulphuric acid. The extract is concentrated and distilled with sodium hydroxide. The distillate is neutralised with hydrochloric acid and evaporated to dryness; the residual hydrochloride is then distilled with solid potassium hydroxide, when the alkaloid passes over as an oil, which can be dried by warming with sodium, and is then distilled in a hydrogen atmosphere.

Sparteine, $C_{15}H_{26}N_2$ (Compare vol. 6, p. 232) is a colourless oil b. p. 311°/723 mm. and 180 to 181°/20 mm., having an odour somewhat like that of aniline, and a very bitter taste. It is readily soluble in most organic solvents, only sparingly in water, and hardly at all in ligroin.

The amount of sparteine in broom tops varies very considerably with the season of the year.

According to Carr and Reynolds (*Pharm. J.*, 1908, [iv], **26**, 542) a number of commercial specimens yielded from 0.07 to 1.06% of sparteine sulphate, and specimens taken throughout a year from the same locality gave a maximum of 0.53% in March, falling to a minimum of 0.07% in August and then slowly rising again. Chevalier (*Compt. Rend.*, 1910, **150**, 1068) has also found a maximum in March and a minimum in August and states that the change is due to the alkaloid wandering into the fruit during the flowering season. The mature seeds may contain as much as 1.1%.

Sparteine sulphate, $B.H_2SO_4.5H_2O$ crystallises from water in long white prisms, m. p. 153°; 1 grm. dissolves in 0.7 c.c. of water and in 6 c.c. of 90% alcohol. This salt is much employed in medicine for its tonic and stimulant action upon the heart.

Sparteine hydrochloride, B,2HCl, white crystals, soluble in water and in alcohol.

Sparteine is a diacid base forming, for instance, a moniodide, $C_{15}H_{26}N_2.HI$, and a di-iodide, $C_{15}H_{26}N_2.2HI$.

The constitution of sparteine has not yet been definitely established, but much light has been thrown on it by the work of Moureu and Valeur who have tentatively suggested the following formula (*Compt. Rend.*, 1905, **141**, 261):

$$CH_2\text{————}CH_2 \qquad CH_2\text{———} CH_2$$
$$CH.CH_2.CH_2.N \qquad N.CH_2.CH_2.CH$$
$$CH_2\text{————}CH.CH_2.CH\text{————}CH_2$$
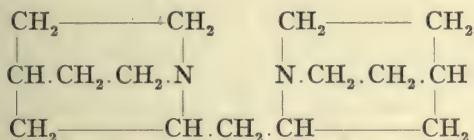
Alkaloids of Sabadilla (*Cevadilla*).

In 1819, the seeds of *Veratrum Sabadilla*,[1] called also *Schœno-caulon officinale* and *Asagraya officinalis*, were found independently by Meissner and by Pelletier and Caventou to contain an alkaloid which was obtained by the former chemists in an amorphous form by boiling the seeds with water acidified with sulphuric acid, and treating the extract with ammonia in excess.

In 1834, Couerbe isolated from a similar product three distinct substances, of which one was amorphous, but yielded a crystallisable sulphate and hydrochloride. It was readily soluble in alcohol and ether, but insoluble in water. To this alkaloid, Couerbe gave the name of *veratrine*. The second alkaloid, called by him *sabadilline*, was insoluble in ether, but soluble in alcohol and water, and was crystallisable therefrom. The third was also soluble in alcohol and water and insoluble in ether, but was amorphous, and formed non-crystalline salts. It was regarded by Couerbe as the monohydrate of sabadilline.

In 1855, Merck obtained from commercial veratrine, by evaporation of a solution in diluted alcohol, a very pure substance, which he succeeded in crystallising. This body, which he called *veratrine*, gave

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[1] The several species of *Veratrum* contain a number of very similar alkaloids, the chemistry and exact relations of some of which are still very obscure. Some of these alkaloids are readily saponifiable, like the aconite alkaloids, which fact adds to the difficulty of their isolation. To increase the confusion, the name "veratrine" has been applied by different observers to several distinct bases, besides indefinite mixtures, and the pharmacopœias of this and other countries describe as "veratrine" the mixed alkaloids obtained from cevadilla seeds. Since cevadine, the principal crystalline alkaloid of cevadilla, is quite different in constitution and properties from jervine, the characteristic alkaloid of white and green hellebore, and the associated alkaloids are also for the most part distinct, it is desirable to consider the alkaloids of sabadilla and the hellebores separately.

upon analysis numbers leading to the formula $C_{32}H_{52}O_8N_2$.   Its salts, with the exception of the aurichloride, failed to crystallise.

In 1871, Weigelin separated three alkaloids from sabadilla seeds. The one identical with Merck's base he considered as capable of existing in two separate forms, one soluble and the other insoluble in water. The other two alkaloids were obtained by precipitating the first base by ammonia and shaking the filtered liquid with fusel oil.

In 1878, Alder Wright and Luff (*Trans.*, 1878, **33,** 338) announced the presence in sabadilla of three distinct alkaloids, all of them saponifiable.   First, an amorphous base, containing $C_{37}H_{53}O_{11}N$, forming a crystallisable sulphate and hydrochloride, and yielding veratric acid, $C_9H_{10}O_4$, on saponification, from which behaviour they named it *veratrine*.   Secondly, the crystallisable alkaloid previously described by Merck, Weigelin, and Schmidt and Köppen, to which they assigned the formula $C_{32}H_{49}O_9N$, and called *cevadine*, because it yielded cevadic acid, $C_5H_8O_2$, on saponification.   And thirdly, an amorphous base, *cevadilline*, containing $C_{34}H_{53}O_8N$, present in very small quantity, and resembling cevadine in yielding cevadic acid on saponification. This base was insoluble in ether, but differed in other respects from the base previously described by Weigelin under the same name.   In 1883, Bosetti concluded that the commercial alkaloid from sabadilla contained two alkaloids which he regarded as isomeric.   For the crystalline base (Wright's cevadine) he retained the name *veratrine*, while he called the amorphous base *veratridine*.   Ahrens and some other recent investigators also use the name "veratrine" to represent *cevadine*, $C_{32}H_{49}O_9N$.[1]   In 1891, E. Merck announced the isolation from sabadilla seeds of two new alkaloids, *sabadine*, $C_{29}H_{51}O_8N$, and *subadinine*, $C_{27}H_{45}O_8N$.

Bearing in mind the foregoing facts, and the circumstance that the different pharmacopœias apply the name "veratrine" to a mixture of the alkaloids of sabadilla, it is desirable to discard this term altogether in its application to a definite chemical individual, and to distinguish the various alkaloids of sabadilla as follows:

Cevadine (Merck's crystalline veratrine), $C_{32}H_{49}O_9N$, crystallisable; on saponification yields cevadic (tiglic) acid, $C_5H_8O_2$, and cevine, $C_{27}H_{43}O_8N$.

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[1] Wright and Luff's nomenclature is adopted in Beilstein's *Organische Chemie*, in the *United States Dispensatory*, the *National Dispensatory*, etc.; while Richter's *Organic Chemistry* still applies the name "veratrine" to Merck's crystalline base, and mentions cevadine as identical with it.

Cevadilline, $C_{34}H_{53}O_8N$, amorphous; on saponification apparently yields cevadic acid, $C_5H_8O_2$, and probably cevine, $C_{27}H_{43}O_8N$.

Veratridine (Couerbe's veratrine), $C_{37}H_{53}O_{11}N$, not crystallisable; on saponification yields veratric acid, $C_9H_{10}O_4$, and verine (possibly identical with cevine).

Sabadine, $C_{29}H_{51}O_8N$, crystallisable.

Sabadinine, $C_{27}H_{45}O_8N$, crystallisable.

The sabadilla alkaloids give the following colour-reactions with strong sulphuric acid:

Cevadine.—Yellow, changing to brownish-yellow and blood-red, with greenish fluorescence. On slight addition of water or prolonged exposure to air the colour changes to purple.

Veratridine.—Reacts exactly like cevadine, except that the red solution is not fluorescent.

Sabadine.—Yellowish, with green fluorescence, changing to blood-red and violet.

Sabadilline.—Permanent blood-red colour.

Commercial "Veratrine."—*Veratrine*, British Pharmacopœia, is "an alkaloid or mixture of alkaloids, prepared from cevadilla". It is described in the British Pharmacopœia of 1898 as answering to the following characters and tests: "Pale grey, amorphous; without odour, but, even in the most minute quantity, powerfully irritating the nostrils; strongly and persistently bitter, and intensely acrid; insoluble in water, soluble in 3 parts of alcohol (90%) or of chloroform, in 6 parts of ether, and in diluted acids, leaving traces of an insoluble brown resinoid matter. It dissolves in nitric acid, yielding a yellow solution. Warmed with hydrochloric acid it dissolves with production of a blood-red colour lasting several days. Treated with fifty or sixty times its weight of sulphuric acid, the mixture turns yellow, subsequently acquires a yellowish-green fluorescence which becomes more distinct on the addition of more acid and slowly changes to bright-red, or, if warmed, violet-red. Heated with access of air, veratrine melts to a yellow liquid, and at length burns away, leaving no appreciable residue (absence of mineral impurities)".

The "veratrine" of the United States Pharmacopœia is described as "a mixture of alkaloids" forming a white or greyish-white amorphous powder. It is said to be soluble in 1,750 parts of water, 2.2 parts of alcohol, 3 parts of ether and in 1 part of chloroform at 25°; soluble in 1,300 parts of water at 80°; very soluble in benzene and amyl

alcohol, insoluble in petroleum benzin. It softens at 145° and melts at 152°.

One part of veratrine mixed with 6 parts of sugar gives a green colouration, changing to blue. The German Pharmacopœia (1910) has the same test (0.01 grm. veratrine + 0.05 grm. sugar) and states that veratrine dissolves in 4 parts of alcohol, in 2·parts of chloroform and in 10 parts of ether. An examination of several samples of commercial "veratrine," which were entirely soluble in ether and otherwise answered the requirements of the German Pharmacopœia, were proved by E. Bosetti (*Arch. Pharm.*; 1883, **221**, 81), to consist of a very intimate, apparently amorphous, mixture of two alkaloids, one of which was crystallisable and insoluble in water ("crystalline veratrine," Wright's "cevadine"), while the other was not crystallisable, but was soluble in water ("veratridine," the "soluble veratrine" of Weigelin, and of Schmidt and Köppen; Wright's "veratrine"). Relatively small quantities of the former alkaloid sufficed to render the latter insoluble in water, while the presence of a small proportion of the latter prevented the crystallisation of the former.

It is evident that commercial "veratrine" is liable to be of very variable quality and physiological activity. If the bases cevadilline, sabadine, and sabadinine be ignored, as occurring in proportions too small to affect materially the character of the article, commercial "veratrine" may be regarded as consisting essentially of cevadine and veratridine, of which cevadine is the more abundant and physiologically active constituent. No quantitative *separation* of the two bases is practicable, but a method of estimating the proportions of each in a mixture of the two could be based on the principle employed by Wright and Luff. Thus the mixture should be boiled with alcoholic sodium hydroxide for a moderate time, half an hour being probably ample. The liquid should then be acidified with dilute sulphuric acid, distilled, and the distillate titrated with dilute sodium or barium hydroxide and phenolphthaleïn. The alkali neutralised by the volatile cevadic or tiglic acid is a measure of the cevadine (and cevadilline) in the sample; while if the veratric or dimethyl-protocatechuic acid be extracted by agitating the contents of the distilling flask with ether, an estimation of the veratridine may be obtained. One c.c. of $N/10$ alkali neutralised by the volatile acid represents 0.0591 grm. of cevadine (and cevadilline) present; and 1 c.c. of similar alkali required by

the acid subsequently extracted by ether corresponds to 0.0667 grm. of veratridine.

Cevadine, $C_{32}H_{49}O_9N$, is the most abundant alkaloid of cevadilla or sabadilla seeds, and, according to Wright and Luff, is also present in the rhizome of *Veratrum viride.* It is identical with the "veratrine" of Merck, and of Schmidt and Köppen, but that name is more appropriately given to the alkaloid first designated thus by Couerbe,[1] which yields veratric acid on saponification, whereas cevadine yields cevadic acid when similarly treated. But the nomenclature is liable to cause great confusion, even recent observers (*e.g.*, Ahrens, Bosetti, Merck) retaining the name "veratrine" for the base which yields cevadic acid, while the pharmacopœias apply the term "veratrine" to the mixture of alkaloids obtained from cevadilla seeds (compare page 69).

Cevadine crystallises from alcohol in anhydrous needles, but from ether it separates only as a varnish, which becomes crystalline on moistening with slightly diluted alcohol and well stirring. The crystals are at first transparent, but on exposure to air become white and opaque, without material loss of weight. The alkaloid melts at 205 to 206°, or at a somewhat lower temperature if impure.

Cevadine dissolves in ethyl acetate, acetone, chloroform, amyl alcohol, and carbon disulphide, but is only sparingly soluble in petroleum spirit, even when hot. The solutions are optically inactive.

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[1] For the extraction of the alkaloids from sabadilla, Alder Wright and Luff (*Trans.*, 1878, **33**, 341) percolated the coarsely pulverised seeds with alcohol acidified with tartaric acid (1 part of acid to 100 parts of seeds), evaporated the liquid to a small bulk, precipitated the resin by adding water, rendered the filtrate alkaline by sodium carbonate, and agitated with ether. The separated ether was then shaken with tartaric acid solution, and employed again. The acid liquid containing the alkaloids as tartrates was again treated with sodium carbonate, and agitated with ether, which completely dissolved the alkaloids. The ethereal solution was cautiously treated with benzoline previously diluted with a little ether until a permanent precipitate began to form, and then set aside, when the ether evaporating the more rapidly, the liquid became gradually richer in benzoline, and deposited first viscid masses of amorphous alkaloid and subsequently distinct crystals. These were stirred up with a few drops of alcohol, well drained, and slightly washed with alcohol on the filter-pump, and the nearly pure crystals of cevadine thus obtained purified by repeated recrystallisation from hot alcohol till they melted at 205°. On treating the viscid amorphous alkaloid with a quantity of ether insufficient for its complete solution, cevadilline remained behind, while on again treating the solution with benzoline and allowing it to evaporate, more cevadine crystallised out. The resinoid precipitate which first separated was dissolved in dilute sulphuric acid, the liquid treated with ammonia, the precipitate drained on the filter-pump, and partially dried by exposure to air. On stirring up the nearly dry base with dilute nitric acid in a mortar, a sticky mass was obtained, which was only partially soluble in water even on boiling. The insoluble portion gradually became granular, and was filtered off and purified by boiling two or three times with small quantities of water. On treating this product with sodium carbonate and ether, evaporating the ethereal liquid, and treating the alkaloidal residue with dilute sulphuric acid, fine crystals of "veratrine" sulphate resembling paper pulp, formed on standing. These were collected and drained on the filter-pump. On spontaneous drying by exposure to air these became a resinoid mass of conchoidal fracture, but on dissolving this in water and allowing the solution to stand, crystals were again formed from which pure "veratrine" (veratridine) was obtained by treating the solution with sodium carbonate and extracting with ether.

Cevadine is extremely poisonous, and exerts a peculiarly powerful action on the mucous membrane of the nose, the smallest particle producing violent sneezing.

Few of the salts of cevadine have been obtained crystallised. On adding auric chloride to a solution of cevadine in hydrochloric acid, the *aurichloride* is thrown down as a very sparingly soluble yellow precipitate, which is amorphous at first, but soon becomes crystalline. When drained and boiled with slightly diluted alcohol it dissolves, and on cooling is deposited in small, well defined needles containing $B,HAuCl_4 + 2H_2O$. The water of crystallisation is lost only slowly at 100°, and the salt melts at 182°. *Cevadine picrate*, $B,C_6H_3O_7N_3$, forms stable crystals, which are very slightly soluble in water, but readily in alcohol, and blacken at 225°. The *mercurichloride*, $B,HCl,HgCl_2$, crystallises in small silvery plates, which melt at 172° with decomposition, are readily soluble in alcohol, but very slightly soluble in water. The *platinichloride* is an amorphous precipitate, soluble in alcohol, but decomposed by water. Alder Wright and Luff found that when cevadine was heated to 100° with twice its weight of benzoic anhydride it was converted into mono-benzoyl-cevadine, $C_{32}H_{48}(C_7H_5O)O_9N$. From the formation of this substance, the impossibility of obtaining a more highly benzoylated derivative, and a study of the products of the saponification of cevadine by alkali hydroxide, Wright and Luff deduced the following constitutional formula for the alkaloid:

$$(C_{27}H_{41}NO_6) \begin{cases} OH \\ OCO\ C(CH_3):CH.CH_3. \end{cases}$$
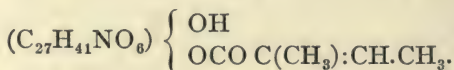
When cevadine is boiled with concentrated hydrochloric acid it yields tiglic acid, $C_5H_8O_2$, and a lustrous ruby-red crystalline mass, which is probably the hydrochloride of a new base. On treatment with nitric acid, cevadine is wholly oxidised; with alkaline potassium permanganate it yields acetic and oxalic acids; and with chromic acid, acetaldehyde and carbon dioxide.

Hydrolysis of Cevadine.—When cevadine is heated in sealed tubes with water to 200° it undergoes hydrolysis. The change occurs more readily when the alkaloid is boiled with alcoholic sodium hydroxide or baryta water, and is also brought about by cold aqueous sodium or potassium hydroxide; and even, though more slowly, by cold dilute ammonia. The first action appears to consist in the for-

mation of angelic acid and a new base called cevine, according to the equation

$$C_{32}H_{49}O_9N + H_2O = C_5H_8O_2 + C_{27}H_{43}O_8N.$$
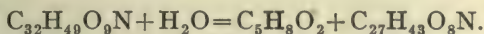
The angelic acid changes with great facility into the isomeric cevadic or tiglic acid, which is to some extent split up into acetic acid, $C_2H_4O_2$, and propionic acid, $C_3H_6O_2$, while the cevine undergoes further decomposition with the formation of non-basic resinous products. The facility with which cevadine undergoes hydrolysis is the cause of the formation of much amorphous alkaloid and other products in the extraction of the alkaloids from cevadilla seeds.

To obtain the two chief products of the saponification of cevadine, Wright and Luff boiled the alkaloid with alcoholic sodium hydroxide under a reflux condenser. The liquid was then diluted with water, acidified with dilute sulphuric acid, and distilled as long as any acid passed over. The distillate was neutralised with soda, evaporated to a small bulk, treated with sulphuric or phosphoric acid, and distilled. The distillate consisted partly of fluid acids, readily soluble in water, and partly of crystals or an oil becoming crystalline on standing. An alternative method is to acidify the solution of the sodium salt with sulphuric acid, and agitate with ether. On distilling the separated ethereal layer after the ether had passed over, an acid liquid began to distil a little above 100°, the temperature quickly rising to 185 to 190°, when a fraction was obtained which solidified on cooling to a mass of crystals wetted by an acid liquid. On pressing this product between blotting-paper, pearly scales of cevadic acid were obtained, m. p. 64 to 65°.

Tiglic acid, Cevadic acid, or *Methyl-crotonic acid*, $CH_3.CH:C(CH_3).$ COOH.—This acid forms anorthic prisms or scales, which melt at 64.5°, though a mixture of it with a somewhat greater weight of its isomeride, angelic acid,[1] is liquid at the ordinary temperature. Tiglic acid has an aromatic odour somewhat resembling butyric acid, but more pleasant, and boils at 198.5°, giving off a vapour which excites violent coughing. When fused with potassium hydroxide, it yields

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[1] **Angelic acid,** stereoisomeric with tiglic acid, crystallises in long prisms, having an aromatic smell, m. p. 44 to 45°, and b. p. 185°. When boiled for some time, or when heated to 100° with sulphuric acid, it is converted into *tiglic acid*. Angelic acid is but slightly soluble in cold water, but dissolves readily in hot water and alcohol, and is extracted from aqueous liquids by agitation with ether. When fused with potassium hydroxide, angelic acid behaves like tiglic acid. *Calcium angelate*, Ca($C_5H_7O_2$)$_2$ + 2H_2O, is much more soluble in cold water than in hot. A cold saturated solution contains about 23% of the salt, but when heated to 30 to 40° glistening needles separate out, and at about 70° the whole becomes semi-solid. If air has been excluded, the crystals re-dissolve completely on cooling.

propionic and acetic acids. *Calcium tiglate*, $Ca(C_5H_7O_2)_2 + 3H_2O$, is soluble in about 16 parts of cold water, but is much more soluble in hot water, and is deposited on cooling in white plates.

Cevine, $C_{27}H_{43}O_8N$.—In order to isolate the complementary alkaloidal product of the hydrolysis of cevadine, Wright and Luff filtered the acid liquid left after distilling off the volatile acid, to separate resinous matter, rendered it alkaline with sodium hydroxide, and agitated with amyl alcohol. The amylic layer, when separated, filtered, and evaporated, yielded a brownish varnish, which, on solution in dilute acetic acid, left resinous flakes. The filtrate from these, on fractional treatment with sodium hydroxide and amyl alcohol, gave an amber-coloured varnish of cevine, perfectly soluble in acids. When heated in a capillary tube, this did not sinter below 140°, and fused at 145°. It dissolved readily in alcohol and amyl alcohol, sparingly in chloroform, and hardly perceptibly in ether. Neither free cevine nor its salts were obtained crystallised. The aqueous solution of cevine becomes turbid on warming. Cevine does not attack the mucous membrane, gives a crimson colour with strong sulphuric acid, and a brown colour with sulphuric acid and sugar.

Freund (*Ber.*, 1904, **37**, 1946) found that when cevine is gently warmed on the water-bath with twice its weight of pure 30% hydrogen peroxide, it is transformed into *cevine oxide*, $C_{27}H_{43}O_9N$, which crystallises well from dilute alcohol and melts at 275 to 278°. This substance may thus be used for characterising cevine; it is an ammonium oxide and is reduced by sulphur dioxide to cevine. Cevine is a tertiary base, but since pyridine does not yield an oxide, and since cevine contains no N-methyl group, Freund supposes the nitrogen to belong to a double cyclic complex, as in hydroberberine.

Veratridine, $C_{37}H_{53}O_{11}N$,[1] occurs in sabadilla seeds, and possibly in minute quantity in the rhizomes of white and green hellebore.[2] It is identical with Wright and Luff's "veratrine."

Veratridine free from cevadine has never been obtained crystallised. It melts at 180°.

On triturating solid veratridine with dilute nitric acid a horny *nitrate* is formed, which is almost insoluble in water, even when boiling. Dilute sulphuric acid readily dissolves veratrine, and, on standing, the *sulphate* crystallises out in extremely slender needles,

[1] The preparation of pure veratridine is described in a footnote on page 73.
[2] See footnote on page 81.

which, on drying, unite to form a horny translucent mass, reproducing crystals when dissolved in water and allowed to stand. The *hydrochloride* exhibits a similar behaviour, but the crystals are not so well marked and distinct. The *aurichloride* is obtained as a gelatinous yellow precipitate, which, when dried over sulphuric acid, becomes a translucent horny mass not crystallisable from alcohol.

When boiled with alcoholic sodium hydroxide veratridine undergoes saponification, with formation of veratric or dimethyl-procatechuic acid and verine, according to the equation

$$C_{37}H_{53}O_{11}N + H_2O = C_9H_{10}O_4 + C_{28}H_{45}O_8N.$$
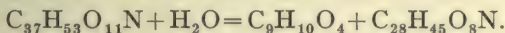
The *acid* is identical with that obtained by the saponification of pseudaconitine (Vol. 6, page 270). The *basic product* verine, or veratroïne, presents a close resemblance to cevine, obtained in a similar manner from cevadine. The only recognisable distinctions observed by Wright and Luff were in the behaviour on heating and the elementary composition. Thus, verine sintered in the water-bath or in a capillary tube at 95°, and on raising the temperature, gradually became a thick viscid mass, exhibiting no definite melting-point, and not becoming completely fluid till heated to about 130°; while cevine exhibited no sign of sintering below 140°, and completely fused at 145°.[1]

Bosetti (*Arch. Pharm.*, 1883, **221**, 81) attributes to veratridine the formula $C_{32}H_{49}O_9N$, and the m. p. 150 to 155°. To the basic product of its saponification he gives the formula $C_{55}H_{92}O_{16}N_2$ and states its m. p. at 143 to 148°.

Sabadine, $C_{29}H_{51}O_8N$, has been isolated from sabadilla seeds by E. Merck (*Chem. Zeit. Rep.*, **15**, 48; *J. Soc. Chem. Ind.*, **10**, 481). It is deposited by the slow evaporation of its alcoholic solution in well-defined crystals, which melt and decompose at 238 to 240°; but the residue obtained by evaporating the ethereal solution has no definite melting point. The crystals are difficultly soluble in water and ether, and insoluble in petroleum-spirit. With strong sulphuric acid, sabadine gives a yellowish colouration and green fluorescence, the colour subsequently changing to blood-red, and finally to violet. Concentrated nitric acid appears to produce no change. The hydrochloride crystallises with

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[1] Wright and Luff, to whom the foregoing observations are due, point out that the numbers obtained by the analysis of veratrine are not incompatible with other formulæ differing but little from $C_{37}H_{53}O_{11}N$, which is that agreeing best with their results. But they add that the formula $C_{36}H_{51}O_{11}N$ would indicate cevine and verine as being identical, and cevadine and veratridine as containing a common radical when their formulæ are thus written:

Veratridine, $(C_{27}H_{42}O_7N)O.CO.C_6H_3(O.CH_3)_2$.
Cevadine,　$(C_{27}H_{42}O_7N)O.CO.C(CH_3):C_2H_4$.

$2H_2O$, but becomes anhydrous and decomposes at 282 to 284°.
Sabadine is not precipitated on adding alkali hydroxides or carbonates
or ammonia to cold solutions of its salts, but is separated in a flocculent
form on warming the liquid.   It can be extracted from the alkaline
liquid by agitation with ether.   Sabadine attacks the mucous mem-
brane of the nose and causes sneezing, but in a less marked degree than
cevadine.

Sabadinine, $C_{27}H_{45}O_8N$, behaves like sabadine with alkalies.   It
is best extracted by agitation with chloroform.   From its ethereal
solution it separates in hair-like needles, which commence to melt at
160°, and decompose at a higher temperature.   The alkaloid is
moderately soluble in water, sparingly in ether, but readily in alcohol.
Concentrated sulphuric acid produces a permanent blood-red coloura-
tion, but nitric acid causes no visible change.   The *hydrochloride* forms
crystals which contain water, and are readily soluble.   Sabadinine
does not occasion sneezing.

Alkaloids of the Hellebores.[1]

The rhizome of white hellebore, *Veratrum album*, was found by
Pelletier and Caventou, in 1820, to contain an alkaloid which they
assumed to be identical with that obtained by them from cevadilla
seeds, and named veratrine.   In 1837, E. Simon confirmed the
presence of veratrine in white hellebore, and discovered a second
alkaloid, *jervine*, readily crystallisable, and remarkable for the in-
solubility of its sulphate.   In 1842, Weigand confirmed the pres-
ence of jervine and veratrine in white hellebore.   Weppen arrived
at the same conclusion in 1872.   In 1872, Peugnet confirmed Simon's
discovery of jervine, but disputed the identity of the second alkaloid
with the veratrine of sabadilla seeds, believing it to be identical with the
base *veratroïdine*, which had been found by Bullock in green hellebore.
Mitchell, in 1874, claimed that this amorphous base was neither
veratrine nor veratroïdine, and called it *veratralbine;* but, in 1876,
Wormley obtained from both white and green hellebore a base which
agreed in all its reactions with the so-called veratrine (cevadine) of
sabadilla.   In 1877, white hellebore was again investigated by Tobien,
who found *jervine*, to which he ascribed the formula $C_{27}H_{47}O_8N_2$,
and an amorphous base, *veratroïdine*, $C_{24}H_{37}O_7N$.   In 1879 (*Trans.*,

1879, **35,** 405), Alder Wright and Luff, as the result of an able in-
vestigation, announced the root of white hellebore to contain the crys-
tallisable alkaloids *jervine*, $C_{26}H_{37}O_3N$; *pseudojervine*, $C_{29}H_{43}O_7N$;
rubijervine, $C_{26}H_{43}O_2N$; an amorphous alkaloid "*veratralbine*" having
a composition approximating to the formula $C_{26}H_{43}O_5N$; and a minute
quantity of "*veratrine*" (veratridine) $C_{37}H_{53}O_{11}N$, to the presence of
which last they attributed the sternutatory properties of the root.
In 1890, C. Pehkschen (*Pharm. Zeit. Russ.*, 1890, **29,** 339; *Jour.
Chem. Soc.*, abst., 1891, **60,** 87) isolated from the rhizome of
white hellebore the alkaloids *veratroïdine*, $C_{32}H_{53}O_9N$; *jervine*, to
which he attributed the formula $C_{14}H_{22}O_2N$; and *pseudojervine*,
$C_{29}H_{49}O_{12}N$. The alkaloids of *Veratrum album* were reinvestigated
(1885 to 1890) by G. Salzberger (*Arch. Pharm.*, 1890, **228,** 462, abst.
Pharm. J., 1891, [iii], **21,** 745, 899), on very large amounts of material.
He confirmed Wright and Luff's descriptions of jervine, pseudojervine,
and rubijervine, but doubted the existence of veratralbine as a chemical
individual. He further isolated two new crystallisable alkaloids, *proto-
veratrine*, $C_{32}H_{51}O_{11}N$, and *protoveratridine*, $C_{26}H_{45}O_8N$, besides a small
quantity of an unnamed crystallisable *base* containing $C_{26}H_{45}O_{10}N$.
Salzberger pointed out that jervine has only a slight toxic action, and
pseudojervine is absolutely inactive. He attributed the sternutatory
property of *Veratrum album* to protoveratrine, which is intensely
poisonous, and excites most violent sneezing; while protoveratridine,
on the other hand, is very bitter, but not poisonous, and is probably
a decomposition-product of protoveratrine.

Green or American Hellebore, *Veratrum viride*, familiarly known as
"Indian Poke," contains in the main the same alkaloids as *Veratrum
album*. In 1838, H. Worthington announced it to contain "an alkaloid
substance identical with *veratrine*." In 1857, J. G. Richardson con-
cluded that "not only in its physical characters, but also in its chemical
actions, the alkaloid of *Veratrum viride* is identical with veratrine of
V. sabadilla." In 1862, J. G. Scattergood announced the presence of
a second alkaloid, insoluble in ether, and a resinous substance to which
the sedative action of the drug was mainly attributable. In 1864, S. R.
Percy extracted from green hellebore an alkaloid which he concluded
had all the properties of veratrine from sabadilla seeds. On the other
hand, in 1865, C. Bullock claimed that the alkaloid of green hellebore
was not identical with veratrine, as it did not respond to the colour-
tests for that alkaloid; that the resin of Scattergood owed its activity

to the presence of another alkaloid; and that these two principles exhibited the same behaviour with mineral acids and with other reagents, but differed in their fusing-points and in their behaviour to ether, in which one was soluble and the other insoluble. For these two alkaloids of green hellebore, G. B. Wood proposed the names *veratroïdine* and *viridine*. In 1872, Peugnet also concluded that the former of these bases was distinct from veratrine, as it did not respond to the sulphuric acid test for that substance, though it did to Trapp's test with hydrochloric acid, and he pointed out that Bullock's viridine was identical with Simon's *jervine* from *Veratrum album*. This was independently proved by C. L. Mitchell in 1874, who prepared jervine from green hellebore, fully described its properties and reactions, and stated that it was not contained in cevadilla seeds. He also obtained a base soluble in ether, which he concluded was distinct from veratrine, as it did not behave like that base with the mineral acids. In 1876, T. G. Wormley also prepared jervine from the roots of green and white hellebore, and concluded that both roots contained an alkaloid which, when pure, fully responded to all the tests for *veratrine*. In the same year, C. Bullock concluded that *jervine* was the only alkaloid in the root of green hellebore, and that the so-called veratroïdine (or, according to Wormley, veratrine) was a mixture of jervine with a light coloured resin, the presence of which rendered the alkaloid more or less soluble in ether. The behaviour of the alkaloid with sulphuric acid, regarded by Wormley and others as due to veratrine, he attributed to a resin soluble in ether, which adhered to the alkaloid with great persistency. Bullock also examined some of the products obtained by Scattergood in 1862, and considered the preparations labelled "veratrine" to consist of jervine mixed with the peculiar resin which produced a mahogany-red colouration with sulphuric acid, and he regarded veratroïdine as of similar composition. These very contradictory observations were reviewed by Alder Wright and Luff, who, in 1879 (*Trans.*, 1879, **35**, 421), reinvestigated the subject, and, by means of improved methods, found the rhizome of *Veratrum viride* to contain the same five alkaloids they had isolated from white hellebore, and, in addition, a base they called *cevadine*, identical with Merck's veratrine from cevadilla seeds.

The proportions of the different alkaloids isolated by Wright and Luff from 1000 parts of the roots of white and green hellebore were as follows:

Alkaloid	Formula	Veratrum album	Veratrum viride
Jervine..............	$C_{26}H_{27}O_3N$	1.3	0.20
Pseudojervine.........	$C_{29}H_{43}O_7N$	0.4	0.15
Rubijervine..........	$C_{26}H_{43}O_2N$	0.25	0.02
Veratralbine..........	$C_{28}H_{43}O_5N$	2.2	traces
Veratridine[1] ("Vera-trine.")	$C_{37}H_{53}O_{11}N$	0.05	less than .004
Cevadine.............	$C_{32}H_{49}O_9N$	apparently absent	0.43
		4.20	0.80

The proportion of total alkaloids obtained by Wright and Luff from green hellebore (= 0.08%) is very much lower than that isolated by other observers. Thus C. L. Mitchell found in three specimens 0.49, 0.61, and 0.69%, of which 0.23, 0.26, and 0.29 consisted of jervine. Bullock obtained 0.66%, while Farr and R. Wright have recorded from 0.16 to 1.20%, with an average of 0.73, of which 0.22 was jervine.

The following table shows the formulæ and leading properties of the characteristic alkaloids of the hellebores, according to the descriptions of Alder Wright and Luff and of G. Salzberger, and, for veratralbine, of C. Pehkschen:

No examination of the rhizome of *Veratrum viride* on the lines of Salzberger's investigation of *V. album* appears to have been made.[2] Dragendorff has found *jervine* in *Veratrum nigrum*, and Tobien (1877) obtained *jervine* and "*veratroïdine*" from the young leaves of *Veratrum lobelianum*.[3]

For the extraction of the total alkaloids from the rhizome of *Veratrum viride*, Farr and Wright (*Chemist and Druggist*, Oct. 29, 1892) recom-

[1] See footnote on page 73.
[2] *Veratrum viride* and *V. album* are commonly called green and white hellebore. They belong to the *Melanthaceæ*, and contain jervine and other well-defined basic principles. On the other hand, *Helleborus fœtidus* and *H. niger*, the black hellebore or Christmas rose, belong to the *Ranunculaceæ*, and contain the poisonous glucosides *helleborin* and *helleboreïn*.
Helleborin, $C_{36}H_{42}O_6$, forms white glittering needles, which, if placed on the tongue, are almost tasteless, but if dissolved in alcohol and then tasted produce a burning, numbing sensation. Strong sulphuric acid dissolves helleborin with intense red colouration, which gradually disappears, a white powder separating. By hydrolysis, helleborin is split into dextrose and helleboresin. Helleborin may be extracted from hellebore root by alcohol, and exhibits toxic properties similar to those of digitalis.
Helleboreïn, $C_{26}H_{44}O_{15}$, forms fine hygroscopic needles, which are bitter and excite sneezing. They are soluble in water and dilute alcohol, but not in ether. Strong sulphuric acid colours helleboreïn golden-yellow, changing to reddish-brown. On hydrolysis, helleboreïn splits into dextrose and helleboretin, a body which when moist is violet-blue, but on drying becomes dirty green. Helleboreïn is present in the seeds and leaves of hellebore, but not in the root.
[3] Robbins (*Proc. Amer. Pharm. Assoc.*, 1877, 439, 523) isolated from green hellebore a crystallised alkaloid which he called veratridine. It possessed a physiological action similar to that of veratrine (cevadine?) though in a less degree. It solution in concentrated sulphuric acid is at first yellow, changing quickly to a pink-red and after standing for some hours assumed a clear indigo-blue colour, very similar to that described by Weppen as yielded by veratrine (cevadine?) if mixed with sugar.

	Jervine	Rubi-jervine	Pseudo-jervine	Proto-veratrine	Proto-veratridine	Veratral-bine (Veratroïdine)
Formula. Crystalline form.	$C_{26}H_{37}O_3N$ Satiny prismatic crystals.	$C_{26}H_{43}O_2N$ Long prisms resembling jervine.	$C_{29}H_{43}O_7N$ Thin hexagonal tables or prisms resembling jervine.	$C_{32}H_{51}O_{11}N$ Rectangular or hexagonal plates or shining prisms.	$C_{26}H_{45}O_8N$ Quadrilateral plates.	$C_{28}H_{43}O_5N$ Amorphous.
Physiological action.	Moderately poisonous.	Inactive.	Inactive.	Very poisonous; violent sneezing.	Bitter; not poisonous.	
Melting-point..	237–242.	240–246.	299–307.	245–250.	265.	149.
Solubility: Alcohol.....	Soluble.	Sparingly.	Sparingly.	Sparingly.	Very sparingly.	Very soluble.
Ether.......	Sparingly sol.	Very sparingly.	Insoluble.	Very sparingly.	Insol.	Readily sol.
Chloroform..	Soluble.	Soluble.	Soluble.	Sparingly.	Very sparingly.	Readily sol.
Petroleum spirit.	Almost insol.	Very slightly.	Very slightly.	Insol.		
Benzene......	Almost insol.	Soluble.	Sparingly.	Insol.	Insol.	Readily sol.
Reaction to litmus.	Alkaline.	Alkaline.	Alkaline.	Alkaline.		
Salts: Sulphate....	Crystalline; nearly insol.	....	Crystalline. Readily sol.	....	....	Amorphous.
Nitrate.....	Crystalline; nearly insol.	....	....	....	....	Amorphous.
Hydrochloride.	Crystalline; very sparingly sol.	....	Very sparingly sol.	....	....	Amorphous.
Precipitants: Auric chloride.	Ppt.; crystalline.	Ppt.	Flocculent ppt.	Amorphous golden-yellow ppt.		
Platinic chloride.	Granular ppt. Pale orange-red.	No reaction.	No reaction.	No reaction.	No reaction.	
Ammonia...:	Fine needles insol. in excess.	Gelatinous ppt.	Cheesy ppt.	On warming, cryst. ppt.	Cryst. ppt.	
Potassiocadmium iodide.	Ppt.	Ppt.	Ppt.	Ppt.	No reaction.	
Mayer's solution.	Ppt.	Ppt.	Ppt.	Ppt.	Ppt.	Ppt. if not too dilute.
Phosphotungstic acid.	Ppt.	Ppt.	Ppt.	Ppt.	Ppt.	
Picric acid..	Ppt.	Ppt.	Ppt.	Ppt.	Ppt.	
Tannin......	Turbidity.	No reaction.	Ppt.	No reaction.	Ppt.	
Colour Tests: Strong sulphuric acid.	Yellow, brownish-yellow, bright green.	Yellow; then orange and dark red.	Yellow; then bright green.	Greenish, blue, and finally violet.	Violet, changing to cherry-red.	Yellow, then organe-red and blood-red, with green fluorescence.
Sulphuric acid and sugar.	Violet, then blue.	....	....	Green, olive-green, dark brown.	....	Brown.
Hydrochloric acid (on warming).	No colouration.	....	....	Cherry-red and odour of isobutyric acid.	Bright red and odour of isobutyric acid.	Rose colour.
Nitric acid..	....	....	....	....	....	Rose, quickly changing to pale yellow.

mend the exhaustion of the drug with 60 to 70% alcohol. Of the
tincture obtained, which, if made of British Pharmacopœia strength,
with 4 oz. of root to the pint of spirit, contains on the average 0.143
grm. of total alkaloid per 100 c.c., 50 c.c. is evaporated in porcelain
at 100°, with addition of water, till the alcohol is driven off. The
residual liquid is slightly acidified with hydrochloric acid, and filtered
through cotton-wool from the precipitated resin into a glass separator.
The separated resinous matter is found to retain alkaloid, and is
therefore redissolved in a little rectified spirit, the solution diluted with
acidified water, the alcohol evaporated, and the liquid filtered into the
separator. The mixed filtrates are rendered distinctly alkaline with
ammonia, and the alkaloid extracted by agitation with chloroform,
using first 10 c.c., and then two successive quantities of 5 c.c. The
chloroform is separated and agitated with successive small quantities
of 1% hydrochloric acid. The acid liquid separated from the chloro-
form is again made alkaline with ammonia, and the alkaloid shaken
out with 15 c.c. of chloroform used in three portions. On evaporating
the separated chloroform and drying the residue at 100°, the veratrum
alkaloids are obtained in a semicrystalline condition, usually entirely
soluble in 2% acetic acid, but occasionally yielding a slightly turbid and
coloured solution, owing to the presence of resinous matter.

Bredemann (*Apoth. Zeit.*, 1906, **21,** 41 to 45 and 53 to 56) estimates
the total alkaloids by shaking 12 grm. of the powdered rhizome with
120 c.c. of a mixture of equal volumes of ether and chloroform, adding
10 c.c. of sodium hydroxide and frequently shaking the mixture
during 3 hours. Water is now added until the powder clogs together
and the ether-chloroform layer is run off, clarified with magnesium
oxide and 3 or 4 drops of water, and 100 c.c. are filtered. The filtrate
is extracted three times with water acidified with acetic acid and after
making the acid extract alkaline, it is shaken up three times with a
mixture of ether and chloroform; after evaporating the solvents, the
residue is dried at 100° and weighed. The percentage of alkaloids was
found by this method to vary from 0.200 to 0.933.

To estimate the *jervine* and distinguish it from the accompanying
alkaloids obtained in their foregoing process, Farr and Wright proceed
as follows: The alkaloidal residue is dissolved in 2% acetic acid, and
filtered, if turbid. A measured quantity of the solution is then treated
with a few grains of potassium nitrate, shaken, and allowed to stand
for some time. The clear liquid is then removed with a pipette, the

crystals washed with a little water, and the latter drawn off when clear. The mixed liquids are measured, made alkaline with ammonia, and agitated with chloroform, which is separated, evaporated, and the residual alkaloid dried and weighed. From the weight obtained, a deduction is made of 0.005 grm. for every 6 c.c. of liquid, as a correction for the solubility of the jervine nitrate, and this correction is added to the weight of jervine obtained by treating the precipitated nitrate with ammoniated water and chloroform, and separating and evaporating the latter.

"The process presents no great difficulty in working, but great care is needed in order to secure perfect extraction of the alkaloids from the chloroformic solution by means of acidified water. This appears to arise from the tenacity with which the resinous matter present adheres to the alkaloid, but may also be due in part to the sparing solubility of the alkaloidal salts produced. In some instances it was found necessary to employ fourteen or fifteen successive portions of acidified water for the shaking-out process before it came away free from alkaloid; it is therefore very important that the process should be repeated until the final washings give no reaction with Mayer's reagent. The process may be considerably shortened by taking the first chloroformic alkaloidal solution, adding 1 c.c. N-hydrochloric acid and 10 c.c. water, evaporating over a water-bath with constant stirring until all chloroform has been removed; filtering from particles of resinous matter, washing the latter with acidified water until the washings come away free from alkaloid; mixing the liquids, making the solution alkaline with ammonia, shaking out the alkaloids with 15 c.c. chloroform added in three portions, drawing off the latter into a dish, and evaporating, drying, and weighing. This modification gives slightly higher results, but the final residue is more highly coloured than that obtained by the original process."—Farr and Wright.

Jervine, $C_{26}H_{37}O_3N$, is the principal crystalline alkaloid of *Veratrum album*, in which Alder Wright and Luff found 1.3%. It is also present in the rhizome and other parts of American or green hellebore, *Veratrum viride*, and according to Tobien, exists in *V. lobelianum* (see footnote, page 81).

The method of isolating jervine from white hellebore root has already been described.

Jervine crystallises from its solution in boiling alcohol in beautiful satiny prismatic needles, generally arranged in tufts, bundles, and

stellar groups, having a characteristic microscopic appearance. The solutions of jervine are slightly lævorotatory.

Jervine melts at 237 to 239°, according to Wright and Luff; at 238 to 242°, according to Salzberger; and at 241° according to Bredemann. It is almost insoluble in water, ethyl acetate, benzene (1:1658), and carbon disulphide, and wholly insoluble in petroleum-ether. It is fairly soluble in acetone and amylic alcohol. At 25° it dissolves in 17 parts of absolute alcohol, and in 60 of chloroform. On spontaneous evaporation of its chloroform solution, jervine is usually obtained as a transparent vitreous mass, which immediately crystallises if touched with a drop of alcohol. Crystallised jervine requires 268 parts of ether for solution, but its solubility is greatly increased by the presence of amorphous alkaloids (e.g., veratralbine), and it is readily extracted by ether from alkaline aqueous liquids.

Jervine is not affected by prolonged boiling with alcoholic potassium hydroxide. It does not produce sneezing, and is only moderately toxic.

Jervine is a well-defined base, having an alkaline reaction to litmus It forms readily crystallisable *salts* with acids. The acetate and phosphate are readily soluble in water, but the hydrochloride, nitrate, and sulphate dissolve very sparingly, and are still less soluble in presence of the corresponding free acids, and hence may be precipitated thereby from their aqueous solutions. They may also be obtained by precipitating a solution of jervine acetate or phosphate with chloride, sulphate, or nitrate of alkali metal. The precipitates rapidly assume a crystalline structure.

Jervine sulphate, $B_2H_2SO_4$, is readily obtained by treating a solution of jervine acetate with excess of dilute sulphuric acid, or by treating free jervine with the dilute acid (1:5). In the first case it is obtained as an immediate precipitate, which rapidly becomes crystalline. In the latter case, the alkaloid does not dissolve, but is converted into an indistinctly crystalline, gelatinous magma, almost insoluble, even after washing, either in cold or boiling water. This property was utilised by Wright and Luff to separate admixed rubijervine, which resembles jervine in being, in presence of amorphous bases, somewhat soluble in ether, while pseudojervine is much less soluble under such circumstances.[1]

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[1] **Rubijervine**, $C_{26}H_{43}O_2N$, is deposited from alcoholic solution in anhydrous crystals, which melt when pure at 236° (Bredemann 234°). It is readily dissolved by dilute hydrochloric acid, but on adding strong hydrochloric acid to the solution the *hydrochloride*

When treated with concentrated sulphuric acid, solid jervine dissolves with yellow colouration, which changes in succession to dark yellow, brownish-yellow and greenish-brown. After standing some time, the mixture assumes a bright green colour, which ultimately disappears, and dirty white or brownish flakes separate, which may become granular or crystalline. The green shades of colour are due to absorption of moisture, and hence, when the test is made in porcelain, the colour is first observable at the edges, and finally extends to the whole liquid, which becomes a dark green. When a test-tube is employed, the green colour is not developed for some hours, but may be immediately produced from the liquid at its greenish-brown stage by slightly diluting with water, the tint becoming successively olive-green, dark chrome-green, and dark emerald, as more water is added. With a further quantity of water the tint becomes lighter, until finally a nearly colourless liquid results, with a few brownish flakes suspended therein. This colour-reaction is peculiar to jervine and pseudojervine. The other alkaloids of hellebore dissolve in cold concentrated sulphuric acid with yellow colour, rapidly becoming brown-yellow, brown, reddish-brown, and finally more or less red in tint; in this respect presenting some resemblance to the behaviour of veratridine and cevadine (page 73).

Sulphuric acid reacts with the sulphate, hydrochloride, and acetate of jervine in the same manner as with the free alkaloid, but it dissolves the nitrate with orange-red colour, which is permanent for several hours.

With Fröhde's reagent, jervine gives a green colouration similar to that produced by sulphuric acid alone.

With sulphuric acid and sugar jervine gives a violet colouration, changing to blue; a reaction said by Pehkschen to distinguish it from veratroïdine (veratralbine), which gives a brown colouration when similarly treated.

separates as a crystalline magma readily soluble in water. The *sulphate* is readily soluble in hot water, or in cold water containing free sulphuric acid, but less readily in cold water free from acid. Rubijervine dissolves in concentrated sulphuric acid to a clear yellow liquid, becoming successively dark yellow, brownish-yellow, and brownish blood-red, changing after several hours to a brownish-purple. On slightly diluting the brownish blood-red liquid with water it becomes successively crimson, purple, dark lavender, dark violet, and ultimately light indigo. Other characters and reactions of rubijervine are given on page 82.

Pseudojervine, $C_{29}H_{43}O_7N$, forms crystals much resembling jervine, but anhydrous, and melting at a considerably higher temperature (299°; Bredemann 304°). The *sulphate* is only sparingly soluble in cold water, but readily in hot water, while the *hydrochloride*, $C_{29}H_{43}O_7N$, $HCl.H_2O$, is only sparingly soluble in ether, or cold or hot water, but is much more readily dissolved by water slightly acidified with hydrochloric acid. Exactly the reverse is the case with jervine and rubijervine hydrochlorides. With strong sulphuric acid, pseudojervine behaves in exactly the same manner as jervine.

Nitric acid dissolves jervine with pinkish colouration to a nearly colourless liquid, which often deposits crystals of the nitrate.

Strong hydrochloric acid gives no colouration with jervine, but immediately converts it into a more or less crystalline hydrochloride, which is insoluble in the acid.

Veratralbine or **Veratroïdine** is the principal amorphous alkaloid of white hellebore root. According to Alder Wright and Luff, the composition approximates to the formula $C_{28}H_{43}O_5N$, while according to C. Pehkschen it contains $C_{32}H_{53}O_9N$, who states that it melts at about 149° and chars at 172°, is optically inactive, and dissolves in alcohol in almost all proportions. It dissolves in 9 parts of ether, 6 of chloroform, or 13 of benzene. According to Salzberger this base is a decomposition-product of protoveratrine (see below) or of other bases. The hydrochloride, hydrobromide, sulphate, nitrate, oxalate, and acetate are amorphous. Veratroïdine gives precipitates with most of the general reagents for alkaloids. With concentrated sulphuric acid, veratroïdine gives a yellow colouration which changes to orange-red and blood-red, with a strong green fluorescence, while concentrated nitric acid produces a transient rose colour, which soon changes to citron-yellow. Hydrochloric acid (11% is the preferable concentration) gives a beautiful rose colouration, which distinguishes the base from veratridine.

With sulphuric acid and sugar veratralbine gives a brown colouration.

The colour-reactions of veratralbine are closely akin to those developed by cevadine, and far more resemble those produced by rubijervine than the reactions yielded by jervine or pseudojervine. Veratralbine does not cause sneezing, and is not changed by prolonged treatment with boiling alcoholic potassium hydroxide.

Protoveratrine, $C_{32}H_{51}O_{11}N$, is described by Salzberger (*Arch. Pharm.*, 1890, **228**, 470) as crystallising from dilute solutions in microscopic, quadrilateral plates, which melt with charring at 245 to 250°. It is the essential active principle of *Veratrum album.*

It is insoluble in water, benzene, and petroleum-spirit, but sparingly soluble in chloroform and boiling alcohol. Cold ether scarcely dissolves it, but when boiling takes up a little more. It is soluble in dilute acids, with the exception of acetic acid. Concentrated sulphuric acid dissolves protoveratrine slowly with green colouration, which passes to cornflower-blue and in some hours to violet. When

warmed with sulphuric acid, the colouration is first light and then dark cherry-red, while an odour of isobutyric acid is evolved.   Concentrated hydrochloric and phosphoric acids produce the same result. With sulphuric acid and sugar, protoveratrine gives an olive-green colouration, becoming dirty green, and finally dark brown.   Solutions of salts of protoveratrine are precipitated by ammonia, Nessler's solution, Mayer's reagent, potassiocadmium iodide, phosphotungstic acid, and picric acid; but not by tannin, platinic chloride, nor mercuric chloride.   The aurichloride is a golden yellow, amorphous precipitate. Protoveratrine is exceedingly poisonous.   A minute amount applied to the nose causes violent sneezing.   The behaviour of protoveratrine with saponifying agents has not been recorded, but the large percentage of oxygen, and the fact that protoveratridine, $C_{28}H_{45}O_8N$, appears to be a decomposition-product, renders it highly probable that it can be hydrolysed.

It is probable that protoveratrine is the sole alkaloid of *Veratrum album* which causes sneezing, as veratridine was not actually isolated therefrom by Wright and Luff, but its existence inferred from the formation of veratric acid.

Protoveratrine can be readily extracted from white hellebore by cold water, but to obtain the crystalline base the rhizome should be freed from fatty and resinous matters by treatment with ether, and then exhausted with alcohol.   The spirit is evaporated in a vacuum, the residue treated with water acidified with acetic acid, rapidly filtered, and the filtrate treated with solid metaphosphoric acid (glacial phosphoric acid), as long as a precipitate is produced.   The liquid is filtered from the   bulky   precipitate   (which   contains   jervine,   rubijervine,   and much amorphous matter), made alkaline with ammonia, and shaken with ether.   The ethereal extract is distilled, when protoveratrine crystallises out, and may be purified from a little jervine and rubijervine by recrystallisation from alcohol.   By this process, Salzberger obtained o.o3% from white hellebore root.   If the ammoniacal solution be subsequently shaken with chloroform, pseudojervine is extracted.

Protoveratridine, $C_{26}H_{45}O_8N$, is a non-poisonous base isolated by Salzberger from white hellebore root.[1]   It crystallises in colour-

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[1] The coarsely powdered rhizome was mixed with water and baryta, and the liquid extracted with ether.   From the solution the ether was evaporated at the lowest possible temperature in a current of hydrogen.   On standing, the resultant dark green syrup gave a crop of crystals mostly consisting of jervine.

less, four-sided plates, m. p. 265°, and is almost insoluble in alcohol, methyl alcohol, acetone, or chloroform, and quite insoluble in ether, benzene, and benzin. It does not cause sneezing, but its solutions in acids are very bitter, and give a crystalline precipitate with ammonia. With concentrated sulphuric acid, the base gives a violet colouration, changing to cherry-red. Its solution in strong hydrochloric acid (like that of veratridine) becomes light red on warming, and evolves a decided odour of isobutyric acid. The solution of the sulphate gives copious precipitates with phosphotungstic, tannic, and picric acids, and with Mayer's reagent. $B_2H_2PtCl_6$ is soluble in water, but is precipitated in large hexagonal plates on adding alcohol to mixed solutions of platinic chloride and protoveratridine hydrochloride.

Alkaloids of the Potato, Etc.

The alkaloid **solanine,** which is at the same time a glucoside, occurs in *Solanum nigrum, S. dulcamara, S. tuberosum* (the potato), etc. Very considerable doubt exists as to the formula of this substance; it seems possible that in various species different alkaloids occur; thus the solanine in *S. sodomæum* of Oddo and Colombano (*Gazzetta*, 1905, [i], **35,** 27) appears to be different from that in the potato.

In the preparation of solanine the use of mineral acids should be avoided, on account of the ease with which the substance is hydrolyzed. The amount present in potato tubers is very small, but the young shoots, formed when the tubers germinate, are much richer in solanine and form a more convenient material for the isolation of the substance.

Firbas (*Monatsh.*, 1889, **10,** 541), macerates the fresh potato shoots with water containing 2% of acetic acid, renders the filtered liquid alkaline with ammonia and extracts the precipitate (after drying) with boiling alcohol, from which the solanine crystallises on cooling.

Cazeneuve and Breteau (*Compt. Rend.*, 1899, **128,** 887) grind the shoots (which should be shorter than 4 inches) with half their weight of lime water, dry the pulp at room temperature and extract with 93% alcohol. On evaporation of the alcohol *in vacuo* a syrup remains, which crystallises on standing. The crystals are washed with ligroin and ether and are recrystallised three times from boiling 95% alcohol. Yield 1 grm. per kilo of shoots.

For the quantitative separation of solanine from potato tubers von Morgenstern (*Landw. Vers. Stat.*, 1907, **65,** 300) grates or pounds

100 to 200 grm. of the fresh material and presses it out under high pressure. The press-cake is twice treated with 200 c.c. of distilled water and pressed again. The three extracts are mixed, acidified with 0.5 c.c. of acetic acid and heated on the water-bath to coagulate proteins. After filtration the solution is evaporated; to the residual syrup 95% alcohol is added until no further precipitate is produced. After standing for 12 hours the precipitate, which consists of starch, gums, etc., is filtered off and washed with hot alcohol. The alcoholic filtrate and washings are evaporated and extracted with water containing a little acetic acid. After filtration the acid extract is heated to the boiling-point, and the solanine in it is precipitated by ammonia. The crude substance so obtained is purified by dissolving in alcohol, filtering, evaporating the alcohol, extracting the residue with water containing a few drops of acetic acid, and again setting free the solanine with ammonia, as described above. The solanine is collected on a filter, tared after heating to 90°, and is dried at that temperature.

Solanine is deposited from hot alcohol in fine colourless, silky needles, which are generally stated to melt (decompose) at about 240°. Cazeneuve and Breteau give the m. p. 250°.

Among the various formulæ assigned to solanine two may be mentioned: $C_{52}H_{93}O_{18}N$, proposed by Firbas (*loc. cit.*) and more recently confirmed by Wittmann (*Monatsh.*, 1905, **26**, 445), and the simpler formula $C_{28}H_{47}O_{10}N$, due to Cazeneuve and Breteau (*loc. cit.*). The chief objection to the former formula is that according to Firbas the action of acids on solanine results in the *liberation* of water:

$$C_{52}H_{93}O_{18}N = C_{40}H_{61}O_2N + 2C_6H_{12}O_6 + 4H_2O.$$
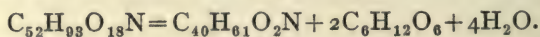
On the other hand, Firbas' formula of the fission product *solanidine* has been confirmed by the analyses and molecular weight determinations of Wittmann. The above equation for the decomposition of solanine cannot be quite accurate, for according to the investigations of Votoček and Vondráček (*Ber.*, 1903, **36**, 4372) and of Zeisel and Wittmann (*ibid.*, 1903, **36**, 3554) both *d*-galactose and rhamnose are formed from solanine; a third sugar, which is perhaps a disaccharide, is also present.

Solanine is odourless when dry, but when moist exhales an odour recalling that of potatoes while cooking. The taste of solanine is somewhat bitter and pungent. It leaves on the pharynx a persistently acrid sensation. Solanine is poisonous, producing in dogs and cats

violent vomiting, followed by somnolence, and sometimes accompanied by paralysis of the lumbar muscles. One grain killed a rabbit in 6 hours, and 1/4 grain is strongly nauseating to a man. Solanine is stated by Sardas to be an excellent neurotic sedative, more efficacious in long-standing neuralgia, especially when neuritis is present, than either antipyrine or antifebrin.

Solanine is nearly insoluble in water, and only very slightly soluble in cold alcohol, but dissolves readily in hot alcohol. It is insoluble in ether, chloroform, benzene, or petroleum-spirit, but is soluble in amyl alcohol, which may be employed to extract solanine from its alkaline solutions.

Solanine is said to have a faintly alkaline reaction. It is a very feeble base, the salts being mostly decomposed by excess of water. The *acid sulphate*, however, is said to be very stable and not decomposed by water, even on heating, in contradistinction to the neutral salt. It is amorphous and very bitter. The *hydrochloride* is precipitated as a jelly on adding ether to a solution of solanine in alcohol acidified with hydrochloric acid. B_2,H_2PtCl_6 is a yellow flocculent precipitate, insoluble in ether, but readily soluble in hot water or in alcohol. The chromate, phosphate, and oxalate of solanine have been obtained crystallised.

Solanine is not affected by alkalies, even when boiling. It does not reduce Fehling's solution, but reduces silver ammonio-nitrate on heating.

Concentrated nitric acid dissolves solanine in the cold to a liquid, which is at first colourless, but rapidly acquires a magnificent purple colour, which soon disappears. With strong hydrochloric acid, solanine gives a yellow colouration. Concentrated sulphuric acid dissolves solanine with orange colour, changing to deep violet and brown.[1]

When warmed with a mixture of equal volumes of alcohol and strong sulphuric acid, solanine dissolves with rose-red colouration. This reaction is stated to be characteristic.

As a micro-chemical test for solanine in plants, Schaarschmidt lays the section to be examined in moderately concentrated sulphuric or nitric acid, when in a few seconds the presence of the alkaloid is indicated by a beautiful rose colour. In this manner, Schaarschmidt recognised the presence of solanine in the tuber and stalk of *Solanum*

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[1] In contradistinction to this, the solanine of Cazeneuve and Breteau was hardly coloured yellow by sulphuric acid (the monohydrate); with fuming nitric acid a pink colour was only developed on long standing. Hydrochloric acid did not colour it.

tuberosum; and also in *S. nigrum, S. dulcamara, Capsicum annuum Lycopersicum esculentum,* and *Mandragora officinalis.*

According to M. E. Wotczal (*Pharm. J.,* 1890, [iii], **21,** 50), with the exception of strong sulphuric acid, only the two following tests are to be relied on for the detection of solanine:

Mandelin's reagent, prepared by dissolving 1 part of ammonium meta-vanadate in 1,000 parts of tri-hydrate of sulphuric acid ($H_2SO_4 + 2H_2O$). With solanine this gives a colour which is first yellow, changing to orange-red, purple-red, brown, pure red, violet, and blue-green, finally disappearing altogether. The reaction is very delicate.

Brandt's reagent, prepared by dissolving 0.3 grm. of sodium selenate in a mixture of 6 c.c. of strong sulphuric acid with 8 c.c. of water. If solanine be warmed with this reagent, the mixture, after cooling, becomes first violet-red, then orange-red and yellow-brown, the colour finally disappearing.

According to Bauer (*Zeit. angew. Chem.,* 1899, 99) telluric acid dissolved in moderately dilute sulphuric acid may be substituted with advantage for the above. The colouration on warming is similar.

Solanidine, $C_{40}H_{61}O_2N$; according to Firbas, $C_{28}H_{41}O_2N$; according to Jorissen and Grosjean (abst. *J. Chem. Soc.,* 1891, **60,** 473) is readily formed from solanine by warming with 2% sulphuric acid; occurs ready-formed in the young sprouts of potatoes in the proportion of 1.5%. It crystallises from alcohol or ether in long silky needles, but is thrown down on adding an alkali to one of its salts as a gelatinous precipitate (sometimes crystalline). Solanidine melts at 208° (191°, Firbas), and sublimes with partial decomposition. It is alkaline to litmus and has a sharp, bitter taste.

Solanidine is very slightly soluble in water, even when hot, but dissolves readily in strong alcohol, and is very easily soluble in ether. It is said to be extracted from its acidulated solutions by agitation with chloroform, and probably with ether also.

Solanidine forms salts which are mostly crystallisable, and sparingly soluble in water and acids. B,HCl forms rhombic prisms with end-faces, can be sublimed, and is readily soluble in alcohol, but very sparingly soluble in water or in hydrochloric acid. B_2,H_2PtCl_6 is yellowish, amorphous, sparingly soluble in water, but readily soluble in acid.

Solanidine is unaltered by treatment with alkalies or dilute acids. It does not reduce either Fehling's solution or silver ammonio-nitrate.

Solanidine dissolves in strong sulphuric acid with red colour, changing to dirty red, the base solanicine, $C_{22}H_{39}ON$, (?) being formed. With alcohol and sulphuric acid it behaves like solanine.

According to Jorissen and Grosjean, if a solution of solanidine in acetic acid be concentrated on the water-bath, hydrochloric acid and a little ferric chloride added, and the mixture then evaporated to dryness, a violet colouration is produced.

In 1883, the occurrence of poisonous symptoms in cattle, after feeding upon the potato-residues from a German distillery, led to an investigation by G. Kassner (*Arch. Pharm.*, 1885, **223**, 243). The residue was treated with ammonia, and then shaken with amyl alcohol, which, when separated and evaporated, left a crystalline residue consisting of solanine and solanidine, the latter predominating owing to the decomposition of the solanine under the influence of the acid mash. The presence of these poisonous alkaloids was attributed to the use either of sprouting or not perfectly ripe potatoes.

Potatoes have also been regarded as the cause of the poisoning of a number of German soldiers, and this occurrence led to estimations of solanine by Gustav Meyer (*Arch. exp. Path. Pharm.*, 1895, **36**, 361) and an investigation of the physiological properties of the alkaloid by Schmiedeberg (*ibid.*, 1895, **36**, 373). Meyer found in unpeeled potatoes 0.0078 to 0.0116%, in peeled ones 0.0040 to 0.0066%. Wintgen (*Arch. Pharm.*, 1906, **244**, 360) showed that solanine is not formed by disease, nor by bacteria, and found in healthy potatoes 0.01 to 0.002% of solanine.

Von Morgenstern (*Landw. Vers. Stat.*, 1907, **65**, 300), using the method already described, found 0.0125% as the average of 18 varieties intended for human consumption. The red varieties contain rather more than the yellow ones. Potatoes grown in a dry sandy soil contain more than those grown in other soils. The solanine content does not increase on keeping, but the substance appears in large quantities during germination and most occurs at the vegetation point.

Alkaloids of Yohimboa Bark.

The bark of a species of *Tabermontana* is employed by the natives of Cameroon as an aphrodisiac. Spiegel (*Chem. Zeit.*, 1896, **20**, 970; 1897, **21**, 833; 1899, **23**, 59) isolated from the bark two alkaloids, the principal of which, *Yohimbine*, is physiologically active.

Yohimbine, $C_{22}H_{28}O_3N_2$, crystallises from dilute alcohol and particularly from benzene in acicular crystals, m. p. 234°, which are very slightly soluble in hot water, and dissolve readily in most organic solvents, but not in cold benzene. Yohimbine forms a colourless solution in concentrated sulphuric acid; when a crystal of potassium dichromate is added a blue-violet colouration is produced, gradually changing to dirty green.

The alkaloid is a monacid tertiary base. The *hydrochloride*, $C_{22}H_{28}O_3N_2,HCl$, m. p. 287°, dissolves fairly readily in water; the *nitrate*, $C_{22}H_{28}O_3N_2,HNO_3$, m. p. 276°, is readily crystallised from hot water. Its solubility in cold water is about 0.9%, in boiling water about 5% (Spiegel, *Ber.*, 1904, **37,** 1762). Yohimbine is the methyl ester of *Yohimboic acid*, $C_{20}H_{26}O_4N_2$, m. p. 269°, which is physiologically inert.

Yohimbenine, $C_{35}H_{45}O_6N_3$, crystalline, m. p. 135°, remains in the yohimbine mother liquors and has no physiological action.

GLUCOSIDES.[1]

By E. FRANKLAND ARMSTRONG, D. Sc., Ph. D., F. C. G. I.

The term *glucoside* is applied to numerous substances possessing the common property of yielding glucose (dextrose) or an analogous compound of the sugar group as one of the products of hydrolysis on treatment with a dilute acid. Thus, salicin when boiled with dilute sulphuric acid is hydrolysed to dextrose and saligenin:

$$\underset{\text{Salicin.}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{Dextrose.}}{C_6H_{12}O_6} + \underset{\text{Saligenin.}}{C_7H_7O.OH.}$$
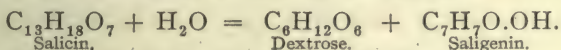
The majority of the glucosides yield ordinary dextro-glucose on hydrolysis; many others yield rhamnoses; others again yield galactose, and a few give remarkable sugars, such as apiose and digitoxose, of abnormal composition. In some cases the nature of the sugar has not been determined with certainty. Only 1 molecule of sugar usually results, though some glucosides contain disaccharides or even trisaccharides and in a few cases yield two different carbohydrates.

The natural glucosides are all products of the vegetable kingdom, but a few substances of animal origin are closely allied to them: these are the paired glucuronic acid derivatives which give rise to glucuronic acid, $CHO.(CH.OH)_4.CO_2H$, instead of dextrose on hydrolysis. The best known of these is euxanthic acid obtained from Purrée or Indian Yellow. A large number of substances when introduced into the animal body are excreted as paired glucuronic acid derivatives.

Some few of the natural glucosides have been prepared synthetically: in addition, a number have been obtained in the laboratory which do not occur in plants.

Hydrolysis is effected in some cases by simply boiling with water under pressure, but it proceeds much more rapidly in presence of an acid. Many glucosides are very resistant to hydrolysis and require prolonged boiling with a fairly strong acid. Most glucosides are wholly unchanged when treated with alkalies, but a few are decomposed.

[1] See also Vol. 1, page 391.

The glucosides are nearly always hydrolysed with relative ease at a low temperature, preferably at about 35° by appropriate enzymes which occur in the plant together with the glucoside. The best-known enzymes are *emulsin* of almonds and *myrosin* of mustard. Some of these enzymes are specific, their influence being exerted only on a few glucosides of closely related composition. Emulsin, however, decomposes a large number of glucosides of very different composition, acting as it were as a master key toward a variety of locks. It has been shown that almond emulsin is a mixture of several allied enzymes; this perhaps explains its catholic action.

The great majority of the glucosides have a bitter taste. As a rule they are neutral or faintly acid, only one or two basic plant principles (*e.g.*, solanine, vernin) being glucosides. Their general properties are largely influenced by the non-sugar constituents, which are of a most diverse nature. Thus, the solubility varies greatly. Most of them are soluble in water and alcohol, insoluble in ether. Many are soluble in ethyl acetate and chloroform. Immiscible solvents also extract them from their acidified acid solutions, a behaviour which affords a general method of separating the glucosides from the stronger alkaloids, though not from weak bases.

They are almost all lævorotatory, though derived from a dextrorotatory sugar. Some of them (digitalin, sapotoxin, strophanthin) are intensely poisonous.

A few glucosides are precipitated by alkaloid reagents, *e.g.*, tannin, picric acid, and some give characteristic colour-reactions with acids, etc. Glucosides are best isolated by extraction with water or dilute alcohol or ethyl acetate after killing the plant enzyme. The solution is clarified with lead acetate, the lead removed and the filtered solution evaporated to the crystallising point. In most cases, owing to the small quantity present, the extraction is a matter of difficulty.

The classification of glucosides is not simple: they are most appropriately grouped according to the nature of the non-sugar component.

The following is a tabular list of the better known glucosides arranged in alphabetical order: in many cases the information available is very scanty and probably needs revision.[1]

[1] Glucosides are dealt with at length in the following special works: Van Rijn, *Die Glucoside*, Berlin, 1900; Roscoe, Schorlemmer's *Chemie, Band* 8, *Pflanzen-glucoside*, Braunschweig, 1901; Armstrong, *The Simple Carbohydrates and Glucosides*, London, 1912; and Thorpe's Dictionary of Applied Chemistry, Vol. 2. Euler und Lundberg. *Biochemisches handlexikon*, Band 2, Berlin 1911.

Name	Chief source	Formula	m.p. °C.	$[\alpha]_D$	Sugar	Other constituents
Aesculin	Aesculus hippocastanum	$C_{15}H_{16}O_9$	205		Dextrose	Aesculetin $C_9H_6O_4$.
Amygdalin	Bitter almonds	$C_{20}H_{27}O_{11}N$	200	−35	Dextrose	Benzaldehyde, hydrogen cyanide.
Amygdonitrile	Cerasus padus, Prunus serotina	$C_{14}H_{17}O_6N$	147	−26.9	Dextrose	d-Mandelonitrile.
Androsin	Apocynum androsaemifolium	$C_{15}H_{20}O_8$	218		Dextrose	Acetovanillone.
α-Antiarin	Antiaris toxicaria	$C_{27}H_{42}O_{10}$	220		Antiarose	Antiarigenin.
Apiin	Apium petroselinum	$C_{26}H_{38}O_{14}$	228		Apiose and dextrose	Apigenin.
Arbutin	Arbutus uva ursi	$C_{12}H_{16}O_7$	187	−65	Dextrose	Quinol.
Aucubin	Aucuba japonica	$C_{13}H_{19}O_8$		−174	Dextrose	Aucubigenin.
Baptisin	Baptisia tinctoria	$C_{25}H_{32}O_{14}$	240	−61	2 Rhamnose	Baptigenin.
Barbaloin	Aloes	$C_{20}H_{18}O_9$			d-Arabinose	Aloemodin.
Calmatambin	Canthium glabrifolium	$C_{19}H_{38}O_{13}$	144	−130	Dextrose	Calmatambetin.
Coniferin	Conifers	$C_{16}H_{22}O_8$	185	−67	Dextrose	Coniferyl alcohol.
Convolvulin	Ipomaea purga	$C_{44}H_{80}O_{27}$	150		Dextrose and rhodeose	Convolvulinolic acid.
Daphnin	Daphne mezereum	$C_{15}H_{16}O_9$	200		Dextrose	Daphnetin.
Datiscin	Datisca cannabina	$C_{21}H_{24}O_{11}$	190		Rhamnose	Datiscetin.
Dhurrin	Sorghum vulgare	$C_{14}H_{17}O_7N$			Dextrose	p-Hydroxymandelonitrile.
Digitalin	Digitalis purpurea	$C_{35}H_{56}O_{14}$	217		Dextrose and digitalose	Digitaligenin.
Digitonin	Ibid	$C_{55}H_{92}O_{28}$	225	−50	2 Dextrose + 2 galactose	Digitogenin.
Digitoxin	Ibid	$C_{41}H_{64}O_{13}$	145		2 Digitoxose	Digitosigenin.
Frangulin	Rhamnus frangula	$C_{21}H_{20}O_9$	228		Rhamnose	Emodin.
Fraxin	Fraxinus excelsior	$C_{16}H_{18}O_{10}$	320		Dextrose	Fraxetin.
Fustin	Rhus cotinus	$C_{36}H_{36}O_{14}$	218		Rhamnose	2 Fisetin.
Gaultherin	Gaultheria procumbens	$C_{14}H_{18}O_8$	100		Dextrose	Methyl salicylate.
Gentiin	Gentians	$C_{25}H_{28}O_{14}$	274		Dextrose and xylose	Gentianin.
Gentiopicrin	Gentians	$C_{16}H_{20}O_9$	191	−198	Dextrose	Gentiogenin.
Glucotropaeolin	Tropaeolum majus	$C_{14}H_{18}O_9NS_2K$			Dextrose	Benzylmustard oil and $KHSO_4$.
Glycyphyllin	Smilax glycyphylla	$C_{21}H_{24}O_9$	175		Rhamnose	Phloretin.
Gossypitrin	Gossypium herbaceum	$C_{21}H_{20}O_{13}$			Dextrose	Gossypetin.
Gynocardin	Gynocardia odorata	$C_{13}H_{19}O_9N_2$	162	+72.5	Dextrose and rhamnose	Hydrogen cyanide and $C_5H_8O_4$.
Hesperidin	Citrus fruits	$C_{50}H_{60}O_{27}$	251		Dextrose and rhamnose	2 Hesperetin.
Incarnatrin	Trifolium incarnatum	$C_{21}H_{30}O_{12}$	242		Dextrose	Quercetin.
Indican	Isatis tinctoria	$C_{14}H_{17}O_6N$	100		Dextrose	Indoxyl.
Iridin	Iris florentina	$C_{24}H_{26}O_{13}$	208		Dextrose	Irigenin.
Jalapin	Convolvulus scammonia, Jalapa orizabensis	$C_{44}H_{66}O_{16}$	131	−23	3 Dextrose	Jalapinolic acid.

Name	Chief source	Formula	m.p. °C	$[\alpha]_D$	Sugar	Other constituents
Kampheritrin	Indigofera arrecta	$C_{27}H_{30}O_{14}$	201	...	2 Rhamnose	Kampherol.
Linamarin	Linum species, Phaseolus lunatus.	$C_{10}H_{17}O_6N$	141	-26	Dextrose	Acetonecyanhydrin.
Lotusin	Lotus arabicus	$C_{28}H_{31}O_{16}N$	...	...	2 Dextrose	Lotoflavin and hydrogen cyanide.
Naringin	Citrus decumana	?	170	...	Dextrose and rhamnose	Naringenin.
Phloridzin	Bark of apple, pear, cherry, plum.	$C_{21}H_{24}O_{10}$	170	...	Dextrose	Phloretin.
Polygonin	Polygonium cuspidatum	$C_{21}H_{20}O_{10}$	202	...	Dextrose	Emodin.
Populin	Poplar	$C_{20}H_{22}O_8$	180	...	Dextrose	Benzoyl saligenin.
Prulaurasin	Prunus laurocerasus	$C_{14}H_{17}O_6N$	122	-52.7	Dextrose	d-Mandelonitrile.
Prunitrin	Prunus emarginata	$C_{22}H_{24}O_{11}$	...	...	Dextrose	Prunetin.
Quercimeritrin	Gossypium herbaceum	$C_{21}H_{20}O_{12}$	247	...	Dextrose	Quercetin.
Quercitrin	Quercus tinctoria	$C_{21}H_{20}O_{11}$	183	-141	Rhamnose	Quercetin.
iso-Quercitrin	Gossypium herbaceum	$C_{21}H_{20}O_{12}$	217	...	Dextrose	Quercetin.
Quinovain	Ladenbergia oblongifolia	$C_{30}H_{48}O_8$	...	$+59$	Quinovose	Quinovaic acid.
Robinin	Robinia pseudacacia	$C_{33}H_{40}O_{19}$	...	...	2 Rhamnose + galactose	Robigenin.
Ruberythrinic acid	Rubia tinctorum	$C_{26}H_{28}O_{14}$	258	...	2 Dextrose	Alizarin.
Rutin	Rue, etc.	$C_{27}H_{30}O_{16}$	184	...	Dextrose and rhamnose	Quercetin.
Sakuranin	Prunus pseudo-cerasu	$C_{22}H_{24}O_{10}$	210	...	Dextrose	Sakuranetin.
Salicin	Willow	$C_{13}H_{18}O_7$	201	-65	Dextrose	Saligenin.
Salinigrin	Salix nigra	$C_{13}H_{16}O_7$	195	-87	Dextrose	m-Hydroxybenzaldehyde.
Sambunigrin	Sambucus nigra	$C_{14}H_{17}O_6N$	151	-76	Dextrose	l-Mandelonitrile.
Saponarin	Saponaria officinalis	$C_{15}H_{14}O_7$	...	...	Dextrose	Saponaretin.
Scopolin	Scopolia japonica	$C_{22}H_{28}O_{14}$	218	...	2 Dextrose	Monomethyl ether of aesculetin.
Serotin	Prunus serotina	$C_{21}H_{20}O_{12}$	245	...	Dextrose	Quercetin.
Sinalbin	Sinapis alba	$C_{30}H_{42}O_{15}N_2S_2$	138	...	Dextrose	Acrinylisothiocyanate and sinapine acid sulphate.
Sinigrin	Brassica nigra	$C_{10}H_{16}O_9NS_2K$	126	...	Dextrose	Allylisothiocyanate+ $KHSO_4$.
Skimmin	Skimmia japonica	$C_{15}H_{16}O_8$	210	...	Dextrose	Skimmetin.
Strophanthin	Strophanthus Kombé	$C_{40}H_{66}O_{19}$	...	...	Rhamnose+ mannose	Strophantidine, methyl alcohol.
Syringin	Syringa vulgaris	$C_{17}H_{24}O_9$	191	-17	Dextrose	Methoxyconiferin.
Vicianin	Vicia angustifolium	$C_{19}H_{23}O_{10}N$	160	-20	Dextrose and arabinose	d-Mandelonitrile.
Xanthorhamnin	Rhamnus species	$C_{34}H_{42}O_{20}$	...	...	Galactose+ 2 rhamnose	Rhamnetin.

Glucosides of Conifers.

Coniferin, $C_{16}H_{22}O_8$, occurs in the cambium sap of coniferous trees, and is found also in beetroot and asparagus. It is readily prepared by evaporating the previously boiled and filtered juice to the crystallising point. It forms white satiny needles, often arranged in stellate groups, which contain $2H_2O$ and effloresce in dry air, become anhydrous at 100°, m. p. 185°. Coniferin is soluble in about 200 parts of cold water, but more readily in hot water and in alcohol. It is insoluble in ether. The aqueous solution of coniferin has a bitter taste, and is lævorotatory ($[\alpha]_D^{20} = -66.9°$). Coniferin dissolves in strong sulphuric acid with red colour, a deep blue resin separating on dilution with water. Moistened with phenol, and then treated with concentrated sulphuric or hydrochloric acid, it rapidly acquires a deep blue colour, the change occurring in sunlight almost instantaneously. By this reaction coniferin can be readily detected in pine wood, and, conversely, pine wood moistened with hydrochloric acid may be used to detect phenols.

Coniferin gives no reactions with metallic solutions, and does not reduce Fehling's solution. Chromic acid mixture oxidises it to vanillin. It is gradually hydrolysed by emulsin into dextrose and coniferyl alcohol. If dilute acid be used as the hydrolysing agent, the coniferyl alcohol becomes polymerised to a resinoid substance.

Syringin, $C_{17}H_{24}O_9 + H_2O$, occurs in the syringa and in the bark of lilac and privet. It crystallises in long slender colourless needles sparingly soluble in cold water, more readily in hot. It becomes anhydrous at 100° and melts at 191°; it has $[\alpha]_D - 17°$. With mineral acids it reacts similarly to coniferin. Emulsin hydrolyses it to dextrose and syringenin, *i.e.*, dimethoxyconiferyl alcohol. The formula of hydrated syringin, $C_{17}H_{26}O_{10}$, is that of the saponins, but it has no relation to these.

Glucosides of Willow and Poplar.

Salicin, $C_{13}H_{18}O_7$, occurs in the bark and leaves of many but not all species of *Salix* (willow) and *Populus* (poplar). It occurs also in the female flowers and in the flower buds of meadow-sweet (*Spirea ulmaria*). The amount varies according to the time of the year.

Salicin is o-hydroxybenzylglucoside, $C_6H_4(OC_6H_{11}O_5).CH_2(OH)$. It is hydrolysed slowly by dilute mineral acids, very readily by the emulsin of almonds and by an enzyme called *salicase* present in willow leaves and twigs, to dextrose and saligenin, $C_7H_8O_2$, which is o-hydroxybenzyl alcohol.

It is prepared by exhausting willow bark with boiling water, concentrating, precipitating impurities with lead acetate equal to 10% of the bark taken, and freeing the filtrate from lead. It is then concentrated *in vacuo* and left to crystallise. The impure salicin is recrystallised from alcohol and decolourised with animal charcoal. It crystallises in needles, plates or rhombic prisms, m. p. 198 to 201°, and decomposes at 240°; in aqueous solution it has $[\alpha]_D^{15} - 65°$.

Salicin has a bitter taste and possesses febrifugal properties. It dissolves sparingly in cold (1:28), very readily in hot water (1:0.7) and dissolves in alcohol (1:30 cold, 1:2 boiling). It is insoluble in organic solvents.

It dissolves in concentrated sulphuric acid with an intense red colouration—this is the so-called "*rutilin reaction.*"

It is not precipitated by tannin, neutral or basic lead acetate or by alkaloid reagents. It gives no colouration with ferric chloride. *Saligenin* crystallises in small tables m. p. 82°. It is soluble in cold water (1:15), alcohol, ether and benzene and is characterised by an indigo-blue colouration with ferric chloride. Ether extracts it from aqueous solutions.

To estimate salicin it should be hydrolysed by emulsin at 37° and the dextrose which is produced estimated. As a rough check the saligenin may be extracted with ether and weighed.

Populin, $C_{20}H_{22}O_8$, or benzoylsalicin occurs in the bark and leaves of the aspen and other poplars and is obtained from salicin on benzoylation. It forms delicate needles, m. p. 180°, and is very sparingly soluble in cold, more soluble in boiling water (1:42). Barium hydroxide hydrolyses it to benzoic acid and salicin, dilute acids form benzoic acid, dextrose and saliretin the condensation product of saligenin. Emulsin is without action. It is coloured bright red by concentrated sulphuric acid.

Salinigrin, $C_{13}H_{16}O_7$, has been found only in *Salix discolor* (Jowett and Potter, *Trans.*, 1900, **77**, 707). It has m. p. 195°, $[\alpha]_D - 87.3°$, and gives no colouration with sulphuric acid. It is an isomeride of helicin since m-hydroxybenzaldehyde is formed on hydrolysis.

Helicin, $C_{13}H_{16}O_7$, the glucoside of *o*-hydroxybenzaldehyde, $C_6H_4(OC_6H_{11}O_5).CHO$, does not occur naturally, but is obtained on oxidising salicin with dilute nitric acid: it was prepared synthetically by Michael from acetochloroglucose and potassium salicylaldehyde. Nascent hydrogen reduces it to salicin. It crystallises in bunches of slender needles, m. p. 174°.

Cyanogenetic Glucosides.

Glucosides yielding hydrogen cyanide on hydrolysis, of which amygdalin is a well-known example, have of late been occasionally found in plants used as fodder, and their detection thus becomes a matter of some importance.

It suffices as a rule to macerate the plant tissue with water at about 30° in a closed vessel and then to remove the hydrogen cyanide formed, by distillation, though owing to frothing the aqueous liquid is often difficult to distil. Hydrogen cyanide can be detected by any of the well-known tests, the formation of a rose-red colouration with sodium picrate paper being among the most sensitive, and estimated quantitatively by the ordinary methods.

The procedure adopted by Henry and Auld[1] (*J. Soc. Chem. Ind.*, 1908, **27,** 428) depends on the partial isolation of the glucoside and its decomposition by boiling with mineral acids. The product is ground as rapidly as possible, weighed, placed in a Soxhlet extraction apparatus and percolated with hot alcohol so as to dissolve out the glucoside. The solvent is distilled off and the residue mixed with 50 c.c. of water and 10 c.c. of 10% hydrochloric or sulphuric acid added. The mixture is then distilled preferably in a current of steam until hydrogen cyanide can no longer be found in the distillate, in which it may be estimated volumetrically by Liebig's method. A slight excess of sodium hydrogen carbonate is preferably added and the solution titrated with an excess of iodine solution. A little of the freshly ground product is macerated with water in presence of an antiseptic to ascertain whether hydrogen cyanide is formed, thereby denoting the presence of the enzyme. By this process Henry and Auld found 0.032 and 0.045% of hydrogen cyanide in two samples of linseed cake, though no acid was formed when the cake was ground with water. Apparently the

[1] Compare Vol. 1, page 392.

enzyme of linseed has been destroyed during the hot expression of the oil so that the manufactured cake is not toxic. Cases are recorded, however, where it has caused cattle poisoning.

Numerous cases of the poisoning of cattle by Java beans (*Phaseolus lunatus*) are on record. Henry and Auld find that dark coloured Java or Mauritius beans yield more hydrogen cyanide than the lighter coloured Burma beans, while the cultivated large white beans produced in Madagascar, France, and the United States usually furnish only traces of the acid. The colour of the beans must not, however, be correlated with the amount of acid that they may produce.

A cyanogenetic glucoside also occurs in the stem and tubers of bitter and sweet cassava. These plants always contain cyanogenetic glucoside and the corresponding enzyme—free hydrogen cyanide is not present.

The best-known cyanogenetic glucosides are:

Amygdalin, $C_{20}H_{27}O_{11}N$, m. p. 200°, is found in bitter almonds and in the kernels of peaches, cherries, plums, apples, etc. It is hydrolysed by emulsin to hydrogen cyanide, benzaldehyde, and 2 molecules of dextrose.

Amygdonitrile glucoside (Prunasin), $C_{14}H_{17}O_6N$, m. p. 147°, was first obtained from amygdalin by the action of dried yeast extract. It has since been found in *Cerasus Padus* and *Prunus serotina*. It has $[\alpha]_D - 26°$ and is hydrolysed to dextrose and *d*-mandelonitrile.

Sambunigrin, $C_{14}H_{17}O_6N$, m. p. 151°, is present in the leaves of the elder, *Sambucus niger*. It has $[\alpha]_D - 76°$ and is hydrolysed by emulsin to dextrose and *l*-mandelonitrile.

Prulaurasin, $C_{14}H_{17}O_6N$, m. p. 122°, occurs in the leaves of the common cherry laurel, *Prunus laurocerasus;* it has $[\alpha]_D - 52.75°$ and yields dextrose and *dl*-mandelonitrile on hydrolysis.

Phaseolunatin, also called *Linamarin*, $C_{10}H_{17}O_6N$, has been obtained from flax, cassava and the beans of *Phaseolus lunatus*. It is hydrolysed to dextrose and acetonecyanhydrin.

Lotusin, $C_{28}H_{31}O_{16}N$, is obtained from *Lotus arabicus*, an Egyptian fodder plant. It is hydrolysed to dextrose, hydrogen cyanide and lotoflavin, a colouring matter of the quercitin group.

Dhurrin, $C_{14}H_{17}O_7N$, is present in the great millet, *Sorghum vulgare*. On hydrolysis dextrose, hydrogen cyanide and *p*-hydroxy-benzaldehyde are obtained.

Gynocardin, $C_{13}H_{19}O_9N$, has been found in *Gynocardia odorata* and *Pangium edule*. It forms dextrose, hydrogen cyanide and a complex substance of the formula $C_6H_8O_4$ when hydrolysed.

Vicianin, $C_{19}H_{25}O_{10}N$, m. p. 160°, $[\alpha]_D$ − 20°, occurs in the seeds of *Vicia angustifolia*. It is decomposed by an enzyme present in certain vetches into hydrogen cyanide, benzaldehyde and a complex sugar *vicianose* which is further hydrolysed by almond emulsin to dextrose and *l*-arabinose (Bertrand, *Compt. Rend.*, 1910, **151**, 325).

Glucosides of Mustard.

Black and white mustard, the seeds of *Brassica* or *Sinapis nigra* and *alba* contain the glucosides *sinigrin* and *sinalbin*, together with an enzyme *myrosin*. They also contain sinapine thiocyanate, the proportion of this being much larger in the white seeds. The commercial ground mustard is a mixture of the two varieties. Both plants belong to the family *Cruciferæ* and are erect annuals.

Black or brown mustard seeds are spherical in shape, very small, about 50 or 60 weighing a grain, of a dark reddish-brown colour outside and yellow within. The surface is reticular and full of small depressions: when crushed and moistened they have a pungent taste and odour.

White mustard seeds are yellow in colour, distinctly larger than the black, about 10 weighing a grain and the surface is smoother. They remain inodourous when crushed and moistened, but have a pungent taste.

Microscopic Structure.—The seed proper consists entirely of minute oil bearing cells which look very like starch granules, but neither give a blue colour with iodine nor polarise light. The husk is very complicated, being built up of no less than six layers. The outer layer is transparent and consists of a single layer of large hexagonal cells, filled with mucilage, which swell up and are ruptured when immersed in water. Beneath this are two layers of large cells the walls of which are thickened at the angles and a single layer of small angular cells, called pali-

sade cells, in the walls of which most of the yellow colour of the husk is
seated.   The inner layers consist of a colourless parenchyma resting
on the aluerone layer of large polygonal cells with fat globules and
granular protein matter.   In the black mustard, the palisade cells are
of unequal length and are superposed with one or two layers of pigment
cells.

A slide of ground mustard shows granular masses of loose fine grey
texture, globular oil drops, here and there patches of the yellow layer
and of the hexagonal mucilage cells.   (Mustard is figured by Clayton,
"A Compendium of Food Microscopy," by Leach, "Food Inspection
and Analysis," and Greenish "Food and Drugs.")

Black mustard seeds contain fixed oil, (31 to 37%), sinigrin and
myrosin and the seed coats contain a soluble mucilage.   Starch is
absent from the ripe seed, though present in unripe mustard.   The
seeds yield about 0.5% of volatile oil.

White mustard seeds contain fixed oil, (25 to 26%), sinalbin, myrosin,
sinapine thiocyanate and mucilage; starch is not present.

Fixed Oil.—The oil of black mustard seed has a brownish-yellow
colour and a mild taste; it closely resembles rape oil in chemical com-
position.   Sp. gr. at 15° 0.916 to 0.920, solidifies at − 17°, saponifica-
tion value 173 to 175, iodine value 96 to 110, and contains the glycerides
of erucic and stearic acids and a liquid fatty acid.   By the lead-salt-
ether method it yields 2.3 to 4% of solid fatty acids.   According to
Lewkowitsch it is used for soap making and is not suitable for burning.

White mustard oil is almost identical with black mustard oil, but
the iodine value is lower (92 to 97) and it yields only traces of solid
acids.   It is used as a burning and lubricating oil.

Both oils have mild rubefacient properties.

Glucosides.—To isolate *sinalbin*, white mustard seed should be
finely ground and carefully freed from fixed oil by pressure or by
extraction with benzene or carbon disulphide.   The dry powder is
then extracted for half an hour with four times its weight of boiling
alcohol and filtered hot; on cooling sinalbin crystallises out.

To prepare *sinigrin*, black mustard seed is similarly extracted with
boiling alcohol, after careful removal of the fixed oil by suitable sol-
vents.   With both varieties of mustard it is advisable to add the pow-
dered seed to the boiling alcohol so as to destroy the myrosin im-
mediately.   The extract is evaporated to dryness and the residue ex-
tracted with cold water.   The aqueous solution is evaporated with

the addition of a little barium carbonate and the residue taken up with boiling spirit. Sinigrin crystallises after a time from this solution. To obtain free myronic acid, sinigrin is mixed with tartaric acid and alcohol; the acid potassium tartrate is removed by filtration and the filtrate evaporated to a syrup. Myronic acid has a strong acid reaction and readily decomposes.

Sinigrin or potassium myronate, $C_{10}H_{16}O_9NS_2K$, crystallises in lustrous colourless needles (m. p. 126°), very soluble in water, sparingly soluble in cold alcohol. It is hydrolysed by myrosin to dextrose, mustard oil or allyl*iso*thiocyanate and potassium hydrogen sulphate.

$$C_{10}H_{16}O_9NS_2K + H_2O = C_6H_{12}O_6 + C_3H_5.NCS + KHSO_4.$$
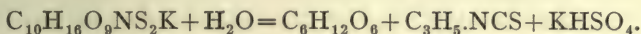
Mustard oil is a mobile volatile liquid with an extremely pungent odour and taste.

Sinalbin, $C_{30}H_{42}O_{15}N_2S_2.5H_2O$, crystallises in faintly yellow coloured needles, m. p. 138 to 140°, slightly soluble in cold, readily in hot water. It is hydrolysed by myrosin to dextrose, sinalbin mustard oil and sinapine hydrogen sulphate.
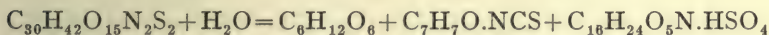
$$C_{30}H_{42}O_{15}N_2S_2 + H_2O = C_6H_{12}O_6 + C_7H_7O.NCS + C_{16}H_{24}O_5N.HSO_4$$

Sinalbin mustard oil is *p*-hydroxybenzyl*iso*thiocyanate; it is not volatile and therefore destitute of pungent odour or effect on the eyes. Sinapine hydrogen sulphate is converted by barium hydroxide into choline and sinapic acid, $C_6H_2(OH)(OMe)_2.CH:CH.CO_2H$.

Sinapine, $C_{16}H_{23}O_5N$, exists as thiocyanate in white (and black) seeds and as sulphate in the glucoside sinalbin. The free base is only known in solution: to obtain it the thiocyanate is treated with silver sulphate and the sulphate precipitated with baryta. It has an intense yellow colour in aqueous solution and is strongly alkaline. On evaporation it decomposes into choline and sinapic acid; with acids it forms crystalline salts. The thiocyanate crystallises in colourless needles (which m. p. 176°) are yellow if impure; it gives a red colouration with ferric chloride.

Myrosin is prepared by extracting ground white mustard seed with cold water and precipitating with strong alcohol. The aqueous extract may be concentrated *in vacuo* below 40° if desired. The precipitated enzyme is soluble in water: the aqueous solution is coagulated

by heat and has the general properties of enzyme solutions. It is very active toward sinigrin and sinalbin. In the case of moderately concentrated solutions of sinigrin, as action proceeds, the potassium hydrogen sulphate formed renders the enzyme less active and finally stops action.

The amount of myrosin contained in black mustard seed is said usually to be insufficient to act on all the glucoside present, while white mustard seed contains an excess of myrosin. It is therefore customary to mix the two varieties[1]. Myrosin has its maximum activity at a little below 50°. It is without action on amygdalin and on phenolic-β-glucosides. Myrosin is widely distributed in cruciferous plants, but the glucosides in the majority of these have been relatively little investigated. The researches of Guignard have shown that myrosin and glucoside are localised in different cells in the seed and are only brought together when the cellular structure is destroyed.

Several other mustard oil glucosides are known.[2]

Mustard is mainly used as a condiment; medicinally its only internal use is as an emetic in poisoning. Externally it is used as an irritant. For local applications the mustard is made up with cold water to a soft uniform cream spread on muslin, cambric or paper and applied directly to the skin; it is kept in contact for 15 to 30 minutes and the skin is then wiped dry. Both black and white mustard are active as sinapisms.

Commercial Mustard.

The mustard of commerce consists of a mixture of the ground seeds of black and white mustard free from hulls which are incapable of the fine grinding necessary to produce a smooth flour. The manufacturer passes the product through a series of sieves which divide it into pure flour and dressings. The yellow hulls are found in the cheaper grades.

The following analyses made by Presse and Stansell in 1880 (*Analyst*, 1880, **5**, 161) show its composition.

[1] Greenish (*Pharm. J.*, 1912, 203) has proved that black mustard seeds contain more than sufficient myrosin.
[2] *Glucotropæolin*, $C_{14}H_{18}O_9NS_2K$, occurs in *Tropæolum majus* and *Lepidium sativum*. It is hydrolysed by myrosin to dextrose, potassium hydrogen sulphate and benzylisothiocyanate.

$$C_{14}H_{18}O_9NS_2K + H_2O = C_6H_{12}O_6 + KHSO_4 + C_7H_7NCS.$$

Gluconasturtium, found in water cress (*Nasturtium officinale*), yields phenylethyliso-thiocyanate on hydrolysis by myrosin.

According to the same authors the ash consists mainly of potassium, calcium and magnesium phosphates, with very little chlorides and no carbonates.

In many cases a large proportion of the fixed oil is removed by subjecting the material to hydraulic pressure—during this process thin hard plates called mustard cake are formed and subsequently reduced to fine powder.

Leach ("Food Inspection and Analysis," page 456) quotes in full the analyses of a number of samples made in his laboratory in 1903 (*J. Amer. Chem. Soc.*, 1904, **26,** 1203). The average of all the commercial varieties of flour tested showed ash 5.03%, ether soluble 18.6%, reducing matter by acid conversion 6.85%. (See also table of results given by Dieterich, in Lunge's "*Chem. Tech. Unt.-Methoden*, 5th Edn. **3,** p. 295.)

Constituents, etc.	White mustard					Brown mustard			
	Whole seeds		Farina			Whole seeds	Farina		
	Yorks	Cambridge	Superfine	Fine	Seconds	Cambridge	Superfine	Fine	Seconds
Moisture............	9.32	8.00	......	5.78	6.06	8.52	4.35	4.52	5.63
Fixed oil...........	25.56	27.51	37.18	35.74	32.55	25.54	36.96	38.02	36.19
Cellulose..........	10.52	8.87	3.90	4.15	9.34	9.01	3.09	2.06	3.26
Sulphur...........	0.99	0.93	1.33	1.22	1.26	1.28	1.50	1.48	1.30
Nitrogen...........	4.54	4.49	5.05	4.89	4.25	4.38	4.94	5.01	4.31
Total proteins......	28.37	28.06	31.56	30.56	26.56	26.50	29.81	30.25	26.06
Soluble albumin and myrosin.	5.24	4.58	7.32	6.67	6.11	5.24	6.46	6.78	6.14
Aqueous extract....	27.38	26.29	36.31	36.60	33.90	24.22	31.64	32.78	31.41
Volatile oil.........	.06	.08	.03	.04	.03	.47	1.44	1.50	1.38
=Potassium myronate............	......	......	......	..	......	1.69	5.14	5.37	4.94
Total ash..........	4.57	4.70	4.22	4.31	4.30	4.98	5.04	4.84	4.91
Soluble ash........	0.55	0.75	0.44	0.55	0.33	1.11	1.01	0.98	0.77

To determine sinigrin and sinapine thiocyanate, Gerard and Duprée digest powdered mustard with a mixture of equal parts of water and alcohol in a reflux apparatus. The extract is evaporated and dried at 105° to constant weight and the residue incinerated so as to transform the acid sulphate into neutral potassium sulphate. The weight of sulphate multiplied by 4.77 gives the weight of sinigrin and this deducted from the total weight of the extract gives that of the sinapine thiocyanate.

It is preferable, however, to estimate sulphate in the ordinary manner and calculate from this.

Cruciferous seeds all yield traces of allyl*iso*thiocyanate or closely allied compounds on treatment with water, but the quantity is very minute, except in the case of black mustard seeds. Thus V. Dircks (*Chem. Soc.*, abstr., 1883, 245) obtained from black mustard-seed cake 1.39% of volatile oil; from yellow mustard cake, 0.018; from rape-seed cake, 0.020 to 0.109 (the proportion of oil apparently decreasing with the age of the cake); from rape seeds, 0.018 to 0.037; from turnip seeds, 0.038; and from the seeds of *Sinapis arvensis*, 0.006% of volatile oil. Mustard-seed cake is highly irritating to cattle, and mustard should be rigidly excluded from cattle foods. Hence the determination of the mustard oil in seed cakes is sometimes of considerable practical importance.

For the **estimation of the pungent volatile oil** in cruciferous seeds and seed cakes several methods have been proposed, depending on the separation of the volatile oil from fixed matters by distillation in a current of steam, and its subsequent conversion into some definite and readily-weighed sulphur compound. The plan usually adopted is to mix the crushed seeds or oil-cake with about ten times its weight of cold or slightly warm water, allowing it to stand for a time varying from half an hour to 6 hours, and then volatilising the oil formed by blowing steam through the flask. V. Dircks (*Landw. Versuchs. Stat.*, **28,** 179), however, insists on the importance of adherence to certain conditions in order to obtain satisfactory results. He recommends that the finely-powdered substance should be mixed with 10 parts of water, and the mixture allowed to stand for 9 hours at 50°, this being necessary to allow of the easy distillation of the oil. Steam and air are then blown simultaneously through the mixture, and the distillate collected in alkaline potassium permanganate, which is subsequently treated with hydrochloric acid and the sulphate formed precipitated as barium sulphate.

Schlicht's modification of Dirck's method is as follows (*Zeit. anal. Chem.*, 1891, **30,** 661): 20 to 25 grm. of mustard flour is mixed to a stiff paste with warm water in a flask through which steam can be blown and to which a condenser is attached. After 30 minutes, steam is blown through the mixture, and the condensed water issuing from the condenser is collected in a receiver (300 c.c.) attached by a cork to the condenser tube, and containing 50 c.c. of a saturated solution

of potassium permanganate, to which is added potassium hydroxide equal in weight to one-fourth of the weight of permanganate present. After 150 to 200 c.c. of water has passed over, the distillate is thoroughly shaken and the excess of permanganate reduced by adding pure alcohol (25 c.c. of alcohol reduce 5 grm. $KMnO_4$), the whole diluted to a known volume and, after filtering through a dry filter-paper, an aliquot portion (preferably one-half) is used for the estimation of sulphate in the ordinary way. In order to oxidise any sulphite that may have been formed by the reducing action of aldehyde, it is necessary to add a little iodine to the measured portion of the solution after acidifying with hydrochloric acid, gradually to raise to the boil and then to precipitate with barium chloride. ($BaSO_4$ found $\times$ 0.4249= mustard oil.)

A later method of Schlicht (*Zeit. öffent. Chem.*, 1903, **9,** 37) is as follows: 25 grm. (or less if much mustard oil be present) is digested with water for 4 hours at the ordinary temperature, in a flask connected with a condenser and a receiver containing potassium permanganate as in the foregoing method. It is then boiled in the same apparatus for 15 minutes, and, after cooling, a solution of myrosin is added and allowed to act for 16 hours. The solution is then steam-distilled and the former procedure carried through. Instead of using myrosin, an alternative method is to digest 25 grm. of the mustard flour with 300 c.c. of water containing 0.5 grm. of tartaric acid for 16 hours at the ordinary temperature, and then to distil with steam.

Mustard oil may also be estimated in the steam distillate by the methods given in Vol. 4, p. 300 *et seq.*

Roeser's method for the estimation of mustard oil in mustard flour (*Analyst*, 1902, **27,** 197) is as follows: 5 grm. of the sample with 60 c.c. water and 15 c.c. of 60% alcohol are set aside for 2 hours.[1] The oil formed is distilled into a flask containing 10 c.c. of ammonia, about two-thirds of the liquid being distilled over. 10 c.c. of $N/10$ silver nitrate are added and the mixture allowed to stand for 24 hours. The solution is made up to 100 c.c. filtered, and 50 c.c. treated with 5 c.c. $N/10$ potassium cyanide. The excess of this is titrated with silver nitrate, using potassium iodide (5%) made slightly ammoniacal as indicator. The number of cubic centimeters of silver nitrate taken up by the oil $\times 2 \times 0.3137$ gives the percentage of mustard oil present.

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[1] This time seems somewhat short.

For other methods see Dieterich (Lunge's "Chem. Tech. Unters. Methoden," Vol. 3, 5th Edn., p. 294; König, *Unters. landw. wichtiger Stoffe);* Vuillemin, *Schweiz. Woch. Chem. Pharm.*, 1904, **43,** 141.

G. Ulex oxidises the sulphur to sulphuric acid with bromine, expels the excess of bromine by heat and decolourises with ammonia.

Volatile oil of mustard, *Allylisothiocyanate*, C_3H_5NCS, is prepared by distillation of the seeds of black mustard after the fixed oil has been expressed and the ground seeds macerated with tepid water for some hours so as to allow the myrosin to hydrolyse the glucoside present.

It is a colourless, mobile liquid with an intensely pungent odour and acrid taste. It is soluble in water (1 in 50), in 70% alcohol (1 in 10), and in organic solvents. D. 1.018 to 1.030, b. p. 147 to 152°. On distillation, the first and last fractions should have the same sp. gr.—this eliminates most adulterations. The crude oil consists almost entirely of allyl*iso*thiocyanate with small quantities of allyl cyanide and carbon disulphide. It should give no colouration with ferric chloride, indicating absence of phenols.

It forms a solid non-volatile compound with ammonia which may be used for its estimation. (See Vol. 4.) On adding excess of ammonia and alcohol to mustard oil the odour disappears and rhombic crystals of thiosinamine or allylthiocarbamide are formed, m. p. 74°. Thiosinamine, $CS.N_2H_3(C_3H_5)$, is readily soluble in water, alcohol and ether, and has a faint leek-like odour.

Volatile oil of mustard is an extremely powerful irritant and causes very rapid vesication when applied to the skin.

The oil is also made artificially by the interaction of allyl iodide and potassium thiocyanate in alcoholic solution: heat converts the allylthiocyanate first formed into mustard oil.

For testing mustard oil for pharmaceutical purposes the following method is that most recently described and it is suggested in place of that given in the fourth edition of the German Pharmacopœia.[1] (See Kuntze, *Arch. pharm.*, 1908, **246,** 58 to 69). 5 c.c. of mustard spirit (2% oil in alcohol), 10 c.c. of ammonia, 50 c.c. of $N/10$ silver nitrate are placed in a 100 c.c. measuring flask, closed with a cork bearing a tube 1 metre long and heated on the water-bath for an hour. The liquid is cooled to 15°, made up to the mark and 50 c.c. of the filtrate

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[1] A similar method is described by Schimmel, *Report*, May, 1906, **45.** See also Vol. 4, page 300.

are made faintly acid with nitric acid and 1 c.c. of ferric ammonium sulphate solution is added. 16.6 to 17.2 c.c. of $N/10$ ammonium thiocyanate should be required to produce a red colouration.

The following are the requirements of the United States Pharmacopœia (8th Revision, 1905):

A volatile oil obtained from black mustard (freed from its fatty oil) by maceration with water and subsequent distillation, yielding, when assayed by the process given below, not less than 92% of allyl isothiocyanate.

A colourless or pale yellow, limpid, and strongly refractive liquid, having a very pungent and acrid odour. *Great caution should be exercised when smelling this oil;* it should not be tasted without being highly diluted. Sp. gr., 1.013 to 1.020 at 25°. Miscible with alcohol in all proportions, forming a clear solution.

If to 3 grm. of the oil 6 grm. of sulphuric acid be gradually added, the liquid being kept cool, the mixture, upon subsequent agitation, will evolve sulphur dioxide, but it will remain of a light yellow colour, and although at first clear, it will afterward become thick and occasionally crystalline, and the pungent odour of the oil will disappear.

If a portion of the oil be heated in a flask connected with a well-cooled condenser, it should distil completely between 148 and 152°, and both the first and the last portions of the distillate should have the same sp. gr. as the original oil (absence of *alcohol, chloroform, petroleum hydrocarbons, fatty oils,* or more than traces of *carbon disulphide*).

If a small portion of the oil be diluted with 5 times its volume of alcohol, and a drop of ferric chloride test solution be added, no blue or violet colour should be produced (absence of *phenols*).

Assay.—Weigh accurately about 2 grm. of volatile oil of mustard, and dilute this with sufficient alcohol to make 50 c.c. of the solution represent 1 grm. of the oil; of this solution, 5 c.c. are transferred to a 100 c.c. measuring flask, and 30 c.c. of $N/10$ silver nitrate and 5 c.c. of ammonia water are added. The flask is well-stoppered and set aside in a dark place for 24 hours. It is then heated in a water-bath, at a temperature of 80°, for half an hour, shaking the flask frequently. The contents of the flask are diluted with water to the 100 c.c. mark and filtered. To 50 c.c. of the filtrate, 4 c.c. of nitric acid and a few drops of ferric ammonium sulphate test solution are added, and finally sufficient $N/10$ potassium sulphocyanate to produce a permanent

red colour; not more than 5.6 c.c. of the latter reagent should be required (each c.c. of $N/10$ silver nitrate consumed corresponding to 0.00492 grm. of allyl isothiocyanate).

Adulterations of Mustard.

Mustard is frequently found adulterated, the most common additions being mustard hulls, wheat flour, turmeric, millet and other weed seed, rice, potato and corn starch. Mineral adulterations are readily detected by an estimation of the ash. The United States standard is 8%. On the other hand, a low ash may indicate admixture with cereals. Wynter Blyth states that the total ash averages 5% and that if below 4 or above 5.5% the mustard is adulterated.

The addition of finely ground hulls to mustard is said to be a common practice and it is difficult to make any restriction as to the amount which should be present. Microscopic examination is the best means of identification and samples in which hulls predominate over the cellular tissue are certainly adulterated. The microscope is also effective in determining adulteration with starches or such substances as wheat bran. Mustard may sometimes naturally contain a little starch, but this amounts at the most to 0.1%.[1] (See Collin, *Annales des Falsifications*, 1909, 206.)

The detection of wheat or other cereal flour in mustard presents no difficulty, but the quantitative estimation is not easy. By exhausting with ether and alcohol, the fixed oil and glucosides may be removed and starch estimated in the residue by any of the ordinary methods. Although mustard hulls have been found to give some reducing matter after treatment with diastase, some workers consider that the direct estimation of starch by the diastase method gives a much more reliable figure than estimations based either on the acid inversion or the proportion of fixed oil.

Estimations based on the proportion of fixed oil were formerly more common. The usual mixtures of black and white seed yield about 35% of fixed oil, so that the percentage of fixed oil as determined by extraction × 2.857 will give the amount of real mustard flour present.

This method is invalidated if any portion of the fixed oil has been

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[1] By the alcoholic potash method from 1 to 2% of something resembling starch is often found.

removed during manufacture and the results will be falsified if oil has been added together with the cereal.

Blyth states mustard contains from 33.9 to 36.7% of oil,[1] wheat-flour from 1.2 to 2.1% and takes the mean value given by the two equations

$$32.7x + 120 = 100y$$
$$34.7x + 200 = 100y$$

where $x = \%$ of mustard in the mixture, $y = \%$ of oil found.

Estimation of the sulphur, which in genuine mustard flour averages about 1.4%, enables any gross adulteration with cereal to be checked. This is conveniently estimated by alkaline permanganate. The mustard flour is boiled with excess of alkaline permanganate and the sulphate formed precipitated by barium chloride. The method of estimation by calculation from the sulphur is not one to be encouraged.

The cheaper grades of mustard flour made from the wild mustard which grows in the wheat fields contain small amounts of wheat and the seeds of various weeds. The commonest of the wild mustards is charlock (*Brassica sinapistrum*) a common weed in corn fields. It contains a dark brown substance in the palisade cells, which on treatment under the microscope with chloral hydrate and gentle heating becomes blood red. Its presence in mustard flour can thus be readily detected.

The addition of wheat flour to mustard is based on the old idea that it is necessary as a preservative and to prevent lumping. In particular the presence of some 1% of flour is claimed to be necessary for mustard which has to stand variations of climate.

Under the Sale of Foods and Drugs Act the addition is permitted only if duly announced by label, such mixed articles being commonly known as mustard condiment.

The United States laws require that "prepared mustard" should contain not more than 12% of crude fibre and not less than 35% of protein on the dry material. Winton and Andrews (Report, *Conn. Expt. Sta.*, 1905, page 123, quoted from Leach, "Food Inspection and Analysis," page 461) give analyses of a large number of American brands. Out of 28, 13 contained cereal flour, 4 salicylic acid and 25 artificial colour.

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[1] F. Sutton states that the amount of fixed oil in the seeds varies according to the soil, climate and locality. Variations from 30 to 47% in the farina of black seeds and of 23 to 38% in white seeds have been observed, but as a rule manufacturers of repute avoid the use of the commoner kinds, which are bad in colour and flavour and are used for making mustard cake for manure.

The A. O. A. C. methods of examining prepared mustard (*U. S. A. Bureau of Chemistry, Bull.* 107, page 167) are as follows:

Prepared Mustard.

1. Preparation of Sample.—The solid portion of the material is commonly in a finely divided condition and does not require grinding, but as it tends to settle, leaving a more or less clear liquid on the surface, thorough mixing is absolutely essential. This may be accomplished by stirring well immediately before removing each portion for analysis.

2. Solids.—Dry 5 grm. to constant weight, in a current of dry hydrogen or *in vacuo* at 100° (see Vol. 1, page 66).

3. Ash.—Burn the dry residue, obtained in the determination of moisture, until free from carbon at as low a temperature as possible (see Vol. 1, page 72).

4. Salt.—Determine chlorine in the ash either gravimetrically or volumetrically. For this purpose the ash is dissolved in nitric acid.

5. Extract.—In a capsule place 10 grm. of the material, about 30 grm. of sand, and a short stirring rod. Heat on a water-bath or in a water oven. Grind until all the lumps are broken up, and determine the ether extract as directed under "VI. General Methods," 39, Bulletin No. 107.

6. Protein.—Determine the nitrogen by the Kjeldahl or Gunning method as directed on page 5, under "I. Fertilizers," Bulletin No. 107. (See Vol 1, page 59.)

7. Acidity.—Weigh 10 grm. into a 200 c.c. graduated flask, make up to the mark with water, shake, filter through a dry paper and determine the acidity in 100 c.c. by titration with $N/10$ alkali, using phenolphthaleïn as indicator. State the results as acetic acid. 1 c.c. of $N/10$ alkali is equivalent to 0.0060 grm. of acetic acid.

8. Copper-reducing Matters Calculated as Starch.—Proceed as directed under the A. O A. C. Method for the determination of starch by acid hydrolysis (Vol. 1, page 420), except that 10 grm. of the material are treated directly with 200 c.c. of water and 20 c.c. of 25% hydrochloric acid, without previous washing or extraction, and the solution is made up to 250 c.c. before filtering and drawing off the aliquot.

9. Crude Fibre.—Weigh 8 grm. of the material (equivalent to about 2 grm. of dry matter) directly into an Erlenmeyer flask and pro-

ceed as directed in Vol. 1, page 70, except that care is taken to add at first only a small amount of 1.25% acid or alkali, and shake thoroughly until all lumps are broken up.   If this precaution is not taken, the lumps will resist the action of the acid or alkali and the results will be high.   Extraction of the fat previous to the treatment is impracticable, as this necessitates preliminary drying, after which the material forms a horny mass.

10. Detection of Colouring Matter.—Proceed as directed in Vol. 5, page 642.

11. Detection of Preservatives.—Proceed as directed in Bulletin 107, p. 179.

Colouring Matter.—Turmeric was at one time largely used with the object of concealing the paleness of tint caused by the addition of flour or other diluent.   It may be detected by the microscope or by the boric acid test after extraction with methylated spirit.

Nitro-dyes, in particular dinitro-α-naphthol (Martius' Yellow) and Naphthol Yellow S are also used; they may be detected by the double dyeing test.

Oil-soluble azo-dyes have been used recently.   They are detected in the same manner as butter colours (see Vol. 5): the ether extract of the sample is shaken with a mixture of 2 parts of carbon disulphide and 15 parts ethyl or methyl alcohol.   The mixture separates into two layers. the carbon disulphide dissolving the oil and natural colour, while the artificial colour remains in the overlying alcohol layer.

Leach draws attention to samples of mustard in which the colour is due largely to the presence of the deep yellow fixed oil which has been restored to the powder after pounding.

A sample recently procured in London contained a yellow colouring matter which turned red with acid.

French Mustard.—Dijon mustard is prepared by grinding the seeds moistened with verjuice made from vinegar or white wine and vinegar, the paste being forced through sieves.   100 kilos of seeds yield about 300 kilos of mustard and 30 kilos of husk.

Cartel (*Annales des Falsifications*, 1909, 215) gives the following analyses:

Fat 10.5%, acetic acid 1.1%, tartaric acid 0.84%, sodium chloride 8.7%, essence of mustard 0.35%.   Eight mustards of the first and second quality contained 0.313, 0.301, 0.288, 0.240, 0.283, 0.323,

0.288, 0.430% of volatile oil of mustard. The amount of acetic acid in these varied from 1.5 to 0.16%—it is usually below 0.8%.

According to the resolution of the manufacturers concerned, only the seeds of *Brassica nigra* may be used for Dijon mustard. Other Cruciferæ are frequently substituted. According to Cartel seeds gathered in 1907 showed the following amounts of volatile oil (essence):

Brassica nigra,	0.9 to 1.3%	
Brassica juncea,	0.85%	
Sinapis glauca,	0.62%	
Sinapis dichotoma,	0.38%	
Brassica rapa,	0.54%	
Brassica napus,	0.23%	(Rape)
Sinapis arvensis,	0.11%	(Charlock)
Eruca sativa,	0.56%	

The recognition of the whole seeds is relatively simple, but in paste form identification is almost impossible. However, as a rule, the cheaper qualities are less finely ground.

Glucosides of Digitalis.

The leaves and seeds of the purple foxglove (*Digitalis purpurea*) contain active principles, many of which are glucosides. The chemical knowledge of digitalis is still far from satisfactory, but the glucosides *digitalin*, *digitoxin*, and *digitonin* have been characterised with some certainty: the nature of *digitalein* and *digitin* is still uncertain. These glucosides are unstable and it is probable that many of the preparations described by various workers in this field really represent mixtures.

The leaves contain digitalin, digitoxin and perhaps digitalein; digitalin and digitonin are present in the seeds. Digitonin is a member of the saponin group and possesses all the properties of these substances. It appears to aid in the solution of some of the other glucosides. Digitoxin possesses the characteritic action of digitalis preparations on the heart and is the most toxic; digitalin is similar but weaker.

Digitalin has the composition $C_{35}H_{56}O_{14}$ or $C_{36}H_{58}O_{14}$; it is a

colourless amorphous powder, m. p. 217°. It is very sparingly soluble in water (1:1,000), readily soluble in hot alcohol and when pure crystallises from the hot saturated solution on cooling in characteristic granular masses. The presence of the other digitalis glucosides prevents this characteristic separation. It crystallises in needles from 85% methyl alcohol. It is sparingly soluble in ether or chloroform.

Concentrated sulphuric acid dissolves it with a golden yellow colouration: this changes to a magnificent rose or violet-red on the cautious addition of potassium hypobromite or other oxidising agent. Thus, with ferric chloride and sulphuric acid the colour is at first an intense golden yellow, then red and changes to a permanent reddish-violet. It is advisable to use only very little digitalin.

It is best hydrolysed by alcoholic hydrogen chloride to avoid the formation of resin and forms dextrose, a sugar, $C_7H_{14}O_5$, termed by Kiliani *digitalose* and *digitaligenin*, $C_{22}H_{30}O_3$, or $C_{23}H_{32}O_3$.

$$C_{35}H_{56}O_{14} = C_6H_{12}O_6 + C_7H_{14}O_5 + C_{22}H_{30}O_3$$
<div align="center">Digitalin.   Dextrose.   Digitalose.   Digitaligenin.</div>
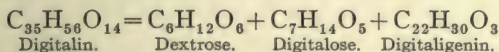
Digitaligenin crystallises from 90% alcohol in granular aggregates of colourless needles, m. p. 210 to 212°.

Digitoxin, $C_{34}H_{54}O_{11}$, crystallises from a mixture of methyl alcohol and chloroform in slender anhydrous prisms which have not melted at 240°, or from dilute alcohol in hydrated crystals, m. p. 145° to 150°. It is insoluble in hot or cold water, soluble in alcohol, chloroform or ether. It gives a characteristic colour reaction when dissolved in glacial acetic acid and concentrated sulphuric acid containing a drop of ferric chloride is cautiously added. A dirty brown or bluish-green band appears at the zone of contact, changing to an intense indigo-blue band. A greenish or brownish-green colouration is obtained on heating with concentrated hydrochloric acid.

It is hydrolysed even at the ordinary temperature by alcoholic hydrogen chloride to digitoxose and digitoxigenin

$$C_{34}H_{54}O_{11} + H_2O = 2C_6H_{12}O_4 + C_{22}H_{32}O_4$$
<div align="center">Digitoxin.   Digitoxose.   Digitoxigenin.</div>
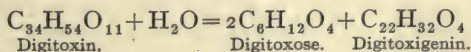
Digitoxose crystallises in prisms or plates, m. p. 102°, and is dextrorotatory. Kiliani has shown it to have the constitution $CH_3.CH(OH).CH(OH).CH(OH).CH_2.CHO$.

Digitoxigenin forms colourless crystals m. p. 230°; alcoholic sodium hydroxide converts it into salts of an acid, $C_{22}H_{34}O_5$, crystallising in bunches of needles m. p. 220 to 230°. By the action of strong hydrochloric acid *anhydrodigitoxigenin*, $C_{22}H_{30}O_3$, is formed: it crystallises in prisms m. p. 215 to 220°.

Digitonin has the composition $C_{54}H_{92}O_{28}$ or $C_{55}H_{94}O_{28}$ and belongs to the saponins. It softens at 225° and melts completely at 235°. The crystals dissolve sparingly in cold water, more readily in hot, to an opalescent solution which froths on agitation. On evaporation, the solution yields only a gummy mass, all attempt to crystallise digitonin from water being hitherto unsuccessful. The aqueous solution is lævorotatory ($[\alpha]_D = -49.25°$), and is precipitated by tannin, ammoniacal lead acetate, and baryta-water. Digitonin is only slightly soluble in absolute alcohol, and still less so in ether, chloroform, or petroleum-spirit. It is almost completely precipitated by adding ether to its alcoholic solution. It crystallises in slender needles from 85% alcohol.

When amyl alcohol is added to an aqueous solution of digitonin, the glucoside is rapidly separated in a crystalline form. If a hot mixture of amyl and ethyl alcohols be used, the solution deposits, on cooling, long nacreous laminæ, which contain amyl alcohol and water of crystallisation. Similar crystalline compounds of digitonin with other alcohols and with phenol have been described by J. Houdas. (See Kiliani, *Pharm. J.*, [iii], **24,** 45).

Digitonin dissolves in strong sulphuric acid with red colour, an addition of a drop of bromine-water intensifying the colour, but not changing it to violet. Sulphuric acid diluted with its own volume of water produces a yellowish colouration in the cold, changing to red and finally to black on heating. Concentrated hydrochloric acid gives a colourless solution, which after a time, or on heating, turns yellow and then violet-red, with a slight greenish fluorescence.

It combines with a molecule of cholesterol in alcoholic solution to give a crystalline precipitate. This reaction affords a valuable test for digitonin and a method for its separation from the commercial digitalin (Windaus, *Ber.*, 1909, **42,** 238). It is hydrolysed on heating with alcoholic hydrogen chloride to dextrose (2 mols.), galactose (2 mols.) and digitogenin, $C_{30}H_{48}O_6$ or $C_{31}H_{50}O_6$, which crystallises in very slender needles, softening at 240°.

The physiological activity of various samples of digitalis varies

enormously and the so-called active principles extracted from the leaf vary even more than the crude preparations. Further, the total amount of glucoside present is of no value as an indication of its activity. The only trustworthy method of standardisation of these and similar drugs is to measure their activity in animals. Dixon ("Manual of Pharmacology," London, 1906, Arnold, which should be consulted for further details) states that digitalis, strophanthus and similar preparations, which have a specific affinity for cardiac muscle, may be tried on the frog's heart and more accurately by perfusing the isolated rabbit's heart with Ringer's solution and subsequently adding the drug.

An infusion and a tincture of digitalis are used in medicine. The infusion contains some digitalin and digitoxin in suspension, though these are insoluble in water, the emulsification being brought about by the digitonin. In old preparations the glucosides decompose, forming resin-like substances which are very toxic.

Digitalis is a cardiac stimulant; it slows the rate of the beat. Externally it is intensely irritant. Given by the mouth it causes vomiting and diarrhœa.

Commercial Digitalin.—The leaves and powder should be dried and kept in sealed vessels, otherwise the activity is rapidly lost. Crushed or powdered leaves are occasionally adulterated with mullein leaves (*Verbascum thapsus*) which are woolly and have branched hairs; primrose leaves which are spatulate and have straight lateral veins; comfrey leaves (*Symphytum officinale*); matico leaves (*Piper angustifolium*), etc.

Digitalinum Germanicum consists chiefly of digitonin and digitalin; it is a yellowish-white amorphous powder soluble in water and alcohol, insoluble in ether and chloroform.

Homolle's amorphous digitalin is amorphous, sparingly soluble in water, readily soluble in alcohol and chloroform. It consists of digitalin with some digitoxin.

Nativelle's crystallised digitalin is mainly digitoxin: it forms fine white needles bitter in taste, insoluble in water, ether or benzene and freely soluble in chloroform.

Keller's method of estimating the digitalis glucosides is that most usually employed. The leaves (20 grm.) are extracted with 70% alcohol,[1] the alcohol evaporated on the water-bath to about 25 grm.

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[1] Extraction is judged to be complete as follows: 3 to 4 c.c. of the percolate is evaporated to dryness, the residue dissolved in 3 c.c. of water to which 2 drops of dilute hydrochloric acid had been added, the solution is filtered and tested with tannin solution: it should show no appreciable turbidity.

and the residue dissolved in water, made up to a weight of 222 grm. and purified with lead acetate (25 grm.). The bulky precipitate is poured onto an 18 cm. filter-paper, and from 132 grm. of the filtrate excess of lead is removed by sodium sulphate (5 grm. sodium sulphate in 7 grm. water). The precipitation is carried out in an Erlenmeyer flask which after shaking well is placed in a sloping position so that the precipitate settles in the angle. After 4 to 5 hours, 130 grm. of the clear liquid (representing 10 grm. of digitalis), can be decanted off entirely clear. This is made alkaline with ammonia (2 c.c. of 10% solution) and extracted several times with 30 c.c. of chloroform each time to remove the digitoxin. The chloroform solution is filtered, evaporated and the residue weighed. It contains fatty and colouring matter besides glucoside and is purified by solution in a minimum of chloroform and precipitation with a mixture of ether (10 c.c.) and light petroleum-spirit (70 c.c.). This purification may be repeated if necessary.

The aqueous solution after extraction with chloroform is made acid and the digitonin precipitated with tannic acid, the tannates are collected, dissolved in 50% alcohol, lead oxide added and the mixture evaporated to dryness. The residue is extracted with dilute alcohol and the extract evaporated.

To the filtrate from the tannic acid precipitation more tannin and concentrated sulphuric acid is added to precipitate the digitalin. (Compare Vanderkleed, *Amer. J. Pharm.*, 1908, **80,** 114, and Burmann, *Bull. Soc. Chim.*, 1910, [iv], **7,** 973.)

By the above method, 4 samples of United States Pharmacopœia tincture gave from 0.023 to 0.037%, 3 fluid extracts gave from 0.234 to 0.264% and the powdered solid extract 1.061% of digitoxin.

The colour reactions of digitoxin and digitalin are summarised by Granier (*J. Pharm. Chim.*, 1908, **27,** 369 to 371). They are:

Keller's Reaction.—(a) 100 c.c. glacial acetic acid and 1 c.c. of 5% ferric sulphate. (b) 100 c.c. strong sulphuric and 1. c.c. of 5% ferric sulphate. The glucoside is dissolved in 1 c.c. of (a) in a narrow tube and 2 c.c. of (b) introduced slowly. Digitoxin gives a bluish-black at the zone of contact and after 2 hours the supernatant acetic acid becomes blue. With digitalin a cherry-red colour is at once formed in the upper stratum of the sulphuric acid.

Lafon's Reaction.—(a) Equal volumes of 95% alcohol and strong sulphuric acid. (b) Very dilute aqueous ferric chloride. A crystal

of the glucoside is moistened with a drop of (a) and a drop of (b) placed in proximity. On contact, digitoxin gives an intense greenish-blue colouration. Digitalin does not afford the reaction. Both these tests can be applied to the evaporated chloroform extract.

Brissemoret-Derrien's Reaction.—(a) 30 c.c. glacial acetic acid mixed with 20 c.c. of 4% oxalic acid reduced to *glyoxalic* acid by treatment with sodium amalgam until neutral. (b) Strong sulphuric acid. The glucoside is dissolved in (a) and 2 c.c. of (b) carefully added. Digitoxin slowly develops a grey or greyish-green tint at the zone of contact. Digitalin gives a cherry-red tint as with Keller's reaction. The glucoside dissolves in (a) with difficulty.

For critical review of analytical methods for digitalis see Focke (*Arch. Pharm.*, 1910, **248**, 365).

Glucosides of Strophanthus.

The seeds of *Strophanthus Kombé*, a climbing plant belonging to the order *Apocynaceæ*, indigenous to eastern tropical Africa, contain the glucoside strophanthin. An extract of the seeds is used by the natives for the preparation of the arrow poison called *Kombé, inée* or *onaje*. The seeds are remarkable for the long *awn* of white silky hairs that is attached to them. These awns are usually removed before exportation and the commercial seeds are of an elongated oval shape 1/2 to 3/4 in. in length and 1/6 in. in thickness. They break easily and in section show 2 straight cotyledons; the odour is characteristic and the taste bitter. A transverse section moistened with sulphuric acid shows a fine green colour.

The commercial drug is often mixed with the seeds of other species of strophanthus.

S. hispidus gives smaller brownish seeds which bear scattered hairs: it gives the green colouration with sulphuric acid.

S. courmonti seeds are smaller, of a more lanceolate shape, less bitter, contain abundant prismatic crystals of calcium oxalate in the seed coat and give a red colouration with concentrated sulphuric acid.

S. nicholsoni has whitish, woolly seeds and gives a red colour with sulphuric acid.

S. gratus has brown and glabrous seeds which give a red colouration with sulphuric acid and contain the glucoside ouabain.

S. emini has greyish-green seeds which contain clusters of calcium oxalate crystals and give a red sulphuric acid colouration.

The genuine seeds are best recognised by their greenish or fawn colour, by the hairs which are satiny not woolly, by the absence of calcium oxalate from the embryo, and by the green sulphuric acid colouration.

The seeds of *S. Kombé* contain about 2 to 3% of strophanthin, about 25% of fixed oil; other constituents are choline, trigonelline and kombic acid, the ash amounting to about 4%.

To isolate the glucoside the freshly powdered seeds are extracted first with ether or carbon disulphide to remove the fat, and then with 70% alcohol. The alcohol is distilled from the extract; the residue dissolved in water, filtered and precipitated with tannic acid, an excess being avoided. The precipitate is washed, mixed with lead oxide, dried, and extracted with alcohol. Ether is added to this alcoholic solution to precipitate the glucoside.

An alternative method suitable for the assay of glucoside is described by Caesar and Loretz (*Pharm. Centralh.*, 1905, **46**, 859). The crushed seeds are extracted for an hour with absolute alcohol, filtered and an aliquot portion evaporated to dryness on the water-bath. The residue is treated with light petroleum spirit to remove the fat, dissolved in boiling water and treated with basic lead acetate solution. The precipitate is collected, well washed and the filtrate freed from lead by means of hydrogen sulphide, evaporated to dryness and the residue, which is crude strophanthin, weighed.

Strophanthin, $C_{40}H_{66}O_{19}$, is a pale yellow amorphous or colourless crystalline powder having an intensely bitter taste and a faintly acid reaction. It melts at 170 to 172° and is remarkable in being dextro-rotatory. It is readily soluble in water. Characteristic is the green colouration obtained with 80% sulphuric acid.

The glucoside is hydrolysed by 0.5% hydrochloric acid at 70° to *strophanthidine hydrate*, $C_{27}H_{38}O_7, 2H_2O$, and *methyl strophantobioside* $C_{13}H_{24}O_{10}$, which latter is in turn further broken down on hydrolysis, yielding rhamnose, mannose and methyl alcohol.

Strophanthidine crystallises in prisms m. p. 235° (anhydrous), 169° (hydrated). It is a dilactone, and Feist (*Ber.*, 1900, **33**, 2069, 2091) has simplified the formula to

$$HO(C_{25}H_{37}O_2){<}\begin{array}{c} CO. \quad O \\ \hline CO. \quad O \end{array}{>}$$
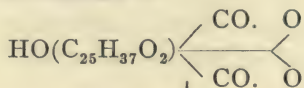
Strophanthin shows certain colour-reactions. On heating with mineral acids it gives various shades of green, changing to violet or blue. A solution of phenol in strong sulphuric acid dissolves it with a violet colouration which subsequently turns green. The addition of a trace of ferric chloride and a little sulphuric acid to the aqueous solution causes a red-brown precipitate. It is precipitated by tannic acid, but soluble in excess.

Strophanthus is intensely poisonous, eight or nine times more so than digitalis. The action on the heart is very similar to that of digitalis, but it has less effect on the nervous system. It is a more effective diuretic than digitalis and a much more powerful muscle poison.

Pseudo-strophanthin, $C_{40}H_{60}O_{16}$, has been found both in *Str. Kombé* (?) and *hispidus*. It is a neutral hygroscopic powder, m. p. 179° and gives a red colouration with sulphuric acid. It is less easily hydrolysed than strophanthin yielding ψ-*strophanthidine*, $C_{27}H_{37}O_6$-CH_3, and a *disaccharide*, $C_{12}H_{22}O_{11}$. ψ-strophanthidine is the methyl ether of a compound containing an oxygen atom less than strophanthidine; it has m. p. 195°. ψ-strophanthin is stated to be more active physiologically than strophanthin. According to the British Pharmacopœia Codex it is probably identical with ouabain obtained from *Str. gratus*.
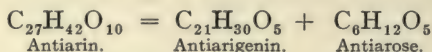
Ouabain, $C_{30}H_{46}O_{12}$, the arrow poison of the Somalis, is contained in the root and wood of *Acokanthera ouabaio* and in the latex of *Strophanthus glaber* (Arnaud, *Compt. Rend.*, 1888, **107**, 1162; 1898, **126**, 346, 1208, 1280, 1684, 1873). It forms slender transparent rectangular plates, m. p. 185°, after previous softening, $[\alpha]_D - 30.6°$. It is sparingly soluble in cold, more readily soluble in warm water, and 85% alcohol. It is insoluble in ether, chloroform and absolute alcohol. It contains water of crystallisation varying according to the temperature from 1 to 2 molecules. It is hydrolysed by dilute acid to a *methyl pentose*—perhaps rhamnose—and a *resin*, $C_{24}H_{36}O_8$, which readily loses 4 molecules of water forming a red resin, $C_{24}H_{28}O_4$.

On heating with alkali, *ouabaic acid*, $C_{30}H_{48}O_{13}$, results: this is a gummy amorphous solid, decomposing at 235°, giving rhamnose on hydrolysis. The literature relating to ouabain has been summarised by R. Müller (*Z. allgem. österr. Apoth. Ver.*, 1908, 46, 319, 331, 343).

Antiarin is the active principle of the latex of *Antiaris toxicaria* of Java. It is used as an arrow poison. It is isolated by adding 1.5

vols. of 95% alcohol to the latex, filtering, evaporating and taking up the residue with water. This solution is precipitated with lead acetate, the filtrate freed from lead and evaporated. Kiliani (*Ber.*, 1910, **43**, 3574) has shown that two glucosides are present in the latex: these he terms α- and β-antiarin.

α-Antiarin crystallises in shining colourless plates, m. p. 220 to 225°, and probably has the composition $C_{27}H_{42}O_{10},4H_2O$. Emulsin is without action on it, acids hydrolyse it with decomposition, the most suitable agent being a mixture of 8 parts 50% alcohol and 2 parts strong hydrochloric acid at 70°.

$$C_{27}H_{42}O_{10} = C_{21}H_{30}O_5 + C_6H_{12}O_5$$
$$\underset{\text{Antiarin.}}{\phantom{C_{27}H_{42}O_{10}}} \quad \underset{\text{Antiarigenin.}}{\phantom{C_{21}H_{30}O_5}} \quad \underset{\text{Antiarose.}}{\phantom{C_6H_{12}O_5}}$$

Antiarose is a methyl-pentose. *Antiarigenin* crystallises in lustrous needles, m. p. 180°: the constitution is not yet known.

β-Antiarin forms slender needles or bunches of columnar needles, m. p. 206 to 207°. It is neutral and has the probable composition $C_{27}H_{38}O_{10}$ or $C_{28}H_{38}O_{10},3H_2O$. It is a glucoside, emulsin is without action on it and the products of acid hydrolysis have not yet been isolated. There is apparently no difference in the toxic character of the two glucosides. Antiarin acts as a muscle and heart poison and closely resembles digitalis.

Windaus and Welsch (*Arch. Pharm.*, 1908, **246**, 504) have obtained a crystalline substance, $C_{39}H_{56}O_2$, from the resin of *A. toxicaria*. This has m. p. 176° and is hydrolysed to α-amyrin and cinnamic acid

Saponins.[1]

The saponins are a group of glucosides, very widely distributed throughout the vegetable kingdom, which possess the common property of forming a clear solution in water, which froths strongly on shaking, forms emulsions with oils and resinous substances, and prevents the deposition of finely divided precipitates. They have a biting taste and in powder form cause sneezing.

The majority of them are homologous substances having the general formula $C_nH_{2n-8}O_{10}$. They vary considerably in the intensity of their toxic action, the more powerful being spoken of as *sapotoxins*. They

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[1] See Kobert's article *Saponins* in Abderhalden's *Handbuch der biochemischen Arbeitsmethoden*, also Blanu, *Dissertation*, Zürich, 1911.

are hydrolysed by dilute acids to dextrose, galactose, pentoses and active substances called *sapogenins*.

They are mostly colloids and not dialysable.

The more important plants which contain active saponins are:

> *Saponaria officinalis*, 4 to 5% sapotoxin.
> *Quillaia saponaria*, 9% sapotoxin.
> *Polygala senega*, 2.5% sapotoxin.
> Sarsaparilla (smilax).
> *Hemidesmus*.
> *Agrostemma githago*, 6.5% sapotoxin.
> *Digitalis purpurea* (digitonin).
> *Claviceps purpurea* (ergotinic acid).

The first two are the most active, senega saponin is only one-eighth as active, and digitonin still less. With the exception of agrostemma they are but little absorbed. They are powerful protoplasmic poisons and are strongly irritant. They are used as expectorants and when administered by the mouth are excreted unchanged. Agrostemma (corncockle) saponin, being absorbed by both the subcutaneous tissue and the intestines, is a dangerous poison.

Saponins are detected in plants by the violet colouration given with concentrated sulphuric acid and the bluish-green colour formed with a mixture of equal parts of alcohol and sulphuric acid with a drop of ferric chloride.

Preparation of Saponins.

Originally hot alcohol was used for extraction, but this gives very impure products. Rochleder, also Christophson, precipitate aqueous extracts with hot saturated barium hydroxide, decompose this precipitate with carbon dioxide and precipitate the glucoside with ether-alcohol. Strütz purifies saponins by conversion into the acetyl derivative, hydrolyses this with barium hydroxide and decomposes with carbon dioxide.

Kobert and Pachorukow add excess of neutral lead acetate to the aqueous extract, whereby acid saponins such as quillaic acid are precipitated. Basic lead acetate is then added to the filtrate to precipitate

the neutral saponins. Both precipitates are washed with alcohol, decomposed with sulphuric acid and the lead removed. The saponin solutions are evaporated, the residue dissolved in alcohol, colouring matters are precipitated by chloroform and the saponins precipitated by ether. Purification either with baryta or by the acetyl method yields physiologically inactive substances for which Kobert suggests the name saponin, reserving sapotoxin for the poisonous substances.

The baryta compound is used for the estimation of saponin. The substance is extracted three times with boiling water, the extract concentrated, precipitated with alcohol and filtered. The precipitate is exhausted with boiling alcohol and added to the filtrate. After the removal of the alcohol the residue is dissolved in water and excess of saturated barium hydroxide solution added. The precipitate is collected, washed, and dried at 100 and 110°. It is weighed, converted by ignition into barium carbonate or into sulphate, and again weighed. This weight is calculated as BaO, and subtracted from the first weight gives that of saponin. When the plant material contains much starch it is advisable to extract with boiling alcohol instead of water. The extract is evaporated and freed from fat with ether.

As a check on the purity of the saponin-baryta precipitate it is dissolved in water containing a little hydrochloric acid, the barium removed quantitatively as sulphate and the filtrate boiled. The sapogenin is collected, washed, dissolved in boiling alcohol (D = 0.855), the solution filtered, evaporated, and dried at 110°. 35.8 parts of sapogenin are taken as equivalent to 100 parts of saponin.

To determine saponins which are not precipitated quantitatively by baryta, May advises the addition of magnesium carbonate and extraction with methyl alcohol. The extract is evaporated, the residue boiled with ethyl alcohol and the saponin precipitated by ether.

The quantitative estimation of the proportion of the products of the hydrolysis of the saponins is valuable as a characteristic. Krushal dissolves in water, adds 2 c.c. hydrochloric acid per 100 c.c., and heats in sealed tubes for 3 hours at 100 to 140°. The sapogenin formed is collected on a tared filter, washed and dried at 100,° carbohydrates being determined in the united filtrate and washings.

Cholesterol is an antidote for saponins, yielding double compounds of varying degrees of dissociation. That of digitonin is crystalline and characteristic (v. this).

The bark of *Quillaia saponaria* (soap bark) contains two toxic glucosides—quillaic acid and sapotoxin.[1]   Commercial saponin is a mixture of these two with inert matter which consists mainly of a non-poisonous modification of quillaic acid and is termed by Kobert saponin.

Quillaic Acid, $C_{19}H_{30}O_{10}$, is prepared by precipitating the aqueous extract of the bark with neutral lead acetate.   The lead is removed from the precipitate, the clear solution evaporated almost to dryness, the residue extracted with boiling absolute alcohol, and chloroform added to precipitate the colouring matter.   The glucoside separates in white amorphous flakes, soluble in water and alcohol, but insoluble in ether.   It becomes dark red on treatment with strong sulphuric acid.

When quillaic acid is purified by the baryta or acetyl processes the physiological activity is entirely lost, but the chemical properties are unchanged.

Quillaia Sapotoxin, $C_{17}H_{26}O_{10}$, is contained in the filtrate from the above lead acetate precipitate.   It is a colourless amorphous powder possessing all the poisonous properties of the sapotoxins and resembles quillaic acid in solubility.   Concentrated sulphuric acid dissolves it with a yellow colouration which slowly becomes yellowish-red.

Saporubrin, $(C_{18}H_{28}O_{10})_4$, the sapotoxin of *Radix Saponariæ rubræ* from *S. Officinalis*, is an amorphous neutral powder which causes sneezing when inhaled.   It tastes at first sweet, but afterward sharp and acid; it is readily soluble in water and dilute alcohol, sparingly so in absolute alcohol.   It dissolves in concentrated sulphuric acid with a reddish-brown colour, becoming reddish-violet on exposure to the air and emerald green on the addition of a few drops of potassium dichromate.   It has $[\alpha]_D - 54°$.

On hydrolysis a series of sapogenins are obtained, those first formed giving up another molecule of sugar on continued action of the dilute acid.   The amount of sugar obtained varies from 60.27 to 63.9%, and of sapogenin from 30.2 to 34.2%, the final product having the formula $C_{14}H_{22}O_2$.

Levant Sapotoxin, $(C_{17}H_{26}O_{10},H_2O)_2$, is obtained from levantine soap wort, *Radix saponariæ albæ*, derived from *Gypsophilla arrosti* or *G. paniculata* by the general method of extraction described on page 125.   The root does not contain a saponin.   The sapotoxin is very

[1] Other constituents of the bark are starch and gum.   The ash is about 8 to 14%.

similar to saporubrin in physiological and chemical behaviour; it dissolves in fuming nitric acid with a yellow colouration, changing to green on the addition of potassium dichromate. On hydrolysis, four molecules of sugar (*i.e.*, 56%), probably a mixture of glucose and galactose, and a *sapogenin*, $C_{10}H_{16}O_2$ (23%), are formed.

Agrostemma-sapotoxin, $(C_{17}H_{26}O_{10})_2$, from the latex of corncockle, *Agrostemma githago*, is a yellowish-white amorphous powder, not precipitated by neutral lead acetate, having the characteristic properties of the group. It yields four molecules of sugar and a sapogenin, $C_{10}H_{16}O_2$, on hydrolysis.

A saponin, $C_{25}H_{42}O_{10}$, from the leaves of *Polyseias nodosa*, is stated by Van der Haar (*Pharm. Zeit.*, 1908, **53**, 900) to yield equimolecular proportions of *l*-arabinose and *d*-glucose on hydrolysis.

Senega root from *Polygala Senega* contains two glucosides—*polygalic acid* which is precipitated by neutral lead acetate, and a sapotoxin *senegin*, $C_{17}H_{26}O_{10}$. These are very similar to the quillaia bark glucosides, but not identical with them. Concentrated nitric acid dissolves polygalic acid with a ruby-red colouration, and senegin with a golden-yellow colouration. The drug contains a small quantity of methyl salicylate which increases as it is kept, probably owing to a gradual decomposition of a glucoside. The drug yields about 4% of ash. Brandl (*Arch. Exp. Path. Pharm.*, 1908, **59**, 245) has shown that the lead acetate precipitate contains a second saponin, *agrostemmic acid*, of higher molecular weight than the sapotoxin, but having the same toxic effects. It yields *sapogenin*, $C_{35}H_{54}O_{10}$, dextrose and galactose, with possibly some arabinose, on hydrolysis. Sarsaparilla, the dried root of various smilax species, contains three saponin glucosides (v. Schulz, *Arb. a. d. Pharmakol. Inst. Dorpath*, 1896, **14**, 14).

Parillin, $C_{26}H_{44}O_{10}$, is a colourless crystalline powder, sparingly soluble in cold water, but soluble in 20 parts of boiling water. It readily dissolves in alcohol of about sp. gr. 0.830. It is obtained from the root by extraction with alcohol and precipitation of the extract with water. The dry powder is colourless, reacts neutral in aqueous solution which froths strongly, and has a sharp bitter taste. It has m. p. 177°, $[\alpha]_D = -42°$, and forms a *pentabenzoyl* derivative, m. p. 76°. On hydrolysis, which is best carried out under pressure, a mixture of two sugars and *parigenin*, $C_{28}H_{46}O_4$, is formed, a colourless crystalline insoluble substance, which on oxidation with nitric acid yields picric, benzoic and oxalic acids.

Smilasaponin, $(C_{20}H_{32}O_{10})_5$, previously called *smilacin* or *sarsaparil saponin*, is obtained by extraction with boiling water and purified by precipitation with baryta. It is a yellowish horny mass which resembles starch flour when powdered. It is lævorotatory. Acids hydrolyse to a *sapogenin*, $C_{28}H_{46}O_4$. Like parillin, it forms a *pentabenzoyl* derivative.

Sarsasaponin, $(C_{22}H_{36}O_{10})_{12}$, is obtained from the aqueous alcoholic mother liquors after precipitation of parillin. It crystallises in long broad needles of silky lustre, m. p. 223°. It is readily soluble in water and in absolute alcohol and forms a *tetra-benzoate*. Acids hydrolyse it to *sarsasopogenin*, $C_{28}H_{46}O_4$. In its chemical behavior it is similar to parillin. Physiologically, sarsasaponin is the most active of the sarsaparilla glucosides, the lethal doses of parillin and smilasaponin being three or four times as large. In addition to the glucosides sarsaparilla contains varying quantities of starch and about 7% of ash. The British Pharmacopœia official drug is known commercially as Jamaica sarsaparilla; other varieties are Lima, Honduras, Guayaquil and Mexican. The Honduras variety is more starchy than the Jamaican and is stated to be preferred on the continent.

Detection of Saponin in Beverages.

Saponins are sometimes added to sparkling wines, lemonade, etc. Kobert's original procedure was to extract with *iso*butylalcohol. Brunner's method (*Z. Unters Nahr. Genussm.*, 1902, **5,** 1197) consists in saturating with magnesium carbonate and extracting with phenol. This is then poured into water and the phenol removed by means of ether. This method has been generally approved (Rühle, *ibid*, 1908, **16,** 165).

Vam Vakas' test (*Z. Unters Nahr. Genussm.*, 1907, **13,** 271) which depends on the formation of a dirty grey coloured precipitate when a saponin solution is treated with a drop of Nessler's reagent has been condemned as not specific by Rosenthaler (*Pharm. Centr.*, 1906, **47,** 587) and by Behre (*Z. Unters Nahr. Genussm.*, 1911, **22,** 498).

Frehse (*Z. Unters Nahr. Genussm.*, 1899, **2,** 938) evaporates the beverage to dryness, extracts the residue with ethyl acetate and tests the extract for a colour-reaction with sulphuric acid. Rühle (l. c.) and Behre (l. c.) have shown that since saponin is almost insoluble in ethyl acetate the method has little value.

Glucosides of Jalap and Scammony.

Jalap and Scammony and several allied plants of the *Convolvulaceæ* yield glucosides of a complicated nature which contain in particular fatty residues. They are mostly drastic purgatives. The active principles of Jalap and Scammony are often miscalled resins.

Convolvulin, $C_{54}H_{96}O_{27}$, is the active principle of true Jalap, the root or tuber of *Ipomœa purga*. It is a colourless, amorphous powder, m. p. 150°, very sparingly soluble in water, but readily soluble in alcohol and acetic acid. Concentrated sulphuric acid dissolves it with a red colouration. It is hydrolysed by mineral acids to dextrose, rhodeose (a methylpentose, $C_6H_{12}O_5$), and *convolvulinolic acid*, $C_{15}H_{30}O_3$. This acid yields on oxidation methylethylacetic acid and *ipomic acid*, $C_{10}H_{18}O_4$, which is an isomeride of sebacic acid.

Jalapin and **Scammonin,** $C_{34}H_{56}O_{16}$, are identical. This glucoside is the active principle of the root stalk of *Ipomœa orizabensis*[1] and of Scammony, the dried sap of *Convolvulus scammonia*. It is an amorphous resinous powder translucent in thin plates, m. p. 131°, $[\alpha]_D - 23°$. It is slightly soluble in water, readily in alcohol, ether, chloroform and benzene. Concentrated sulphuric acid dissolves jalapin with a purple or maroon colour, changing to brown and finally becoming black. Acids hydrolyse it to dextrose (3 mols.) and *jalapinolic acid*, $C_{16}H_{30}O_3$: this acid yields methylethylacetic acid, sebacic acid and an isomeride of this on oxidation.

Turpethin, $C_{34}H_{56}O_{16}$, from the dried sap or root of *Ipomœa turpethum*, is very similar to jalapin, but differs in being insoluble in ether. It is an amorphous powder, m. p. 149°.

Jalap.

Commercial jalap consists of the dried tubercules of *Ipomœa purga*. It occurs in pieces about the size of an egg which, however, vary much in size (2.5 to 7.5 cm.) and shape, the larger roots being cut. Externally they are dark brown, furrowed and wrinkled and are marked with transverse scars. They break with difficulty. The internal surface is yellowish-grey or dull brown, and a transverse section shows concentrically arranged irregular dark lines. The drug tastes at first sweet, then acrid and disagreeable.

[1] The recent work of Power and Rogerson throws doubt on the existence of jalapin as a chemical individual (see p. 133, note).

The principle constituent is the glucoside resin, but the root also contains sugar, starch, proteins and calcium oxalate present in the form of rosette cluster crystals. In other convolvulaceous roots the calcium oxalate crystals are generally acicular (Greenish), a character which may be used to distinguish jalap. The drug yields about 6% of ash. The amount of resin varies from 5 to 18% or generally from 8 to 12%. According to the British Pharmacopœia it should yield not less than 9 nor more than 11%. Jalap, United States Pharmacopœia, should contain not less than 8% total and not more than 1.5% ether soluble resin. Greenish states that the quality of the drug has deteriorated during the last twenty-five years, 12 to 18% being formerly the usual amount of resin present.

Samples analysed by Evans, Sons, Lescher and Webb in 1907 to 1910 contained from 7.4 to 11% of resin. The tubers usually contain from 12 to 16% of moisture. They extract the resin from the drug in 60 powder by boiling with 95% alcohol.

Moore (*J. Soc. Chem. Ind.*, 1904, 412) gives the average resin content of 98 samples of jalap (bought in America) as 12.6%. Subsequently (*J. Soc. Chem. Ind.*, 1906, 627) 276 samples were examined only 15 of which were equal to or above 11%. The average was 5.95% of resin, showing that low grade jalap is of frequent occurrence.

The glucoside resin is a mixture of 2 glucosides, one, about 10% of the whole, being soluble in ether, and the other, *convolvulin*,[1] about 90% of the whole, being insoluble in ether, but soluble in alcohol. The soluble resin is identical with that from scammony root and is termed scammonin or jalapin.

Tampico Jalap, the root of *I. simulans*, is distinguished by its irregular shape and convoluted surface, which does not show the transverse scars characteristic of true jalap. It contains about 10% of ether soluble resin (scammonin).

Oriziba jalap (Mexican scammony), the root of *I. orizabensis*, occurs mostly as transverse slices 5 to 10 cm. long and 1.5 to 2 cm. thick, evidently portions of a large root divided. It contains 17 to 18% of resin (scammonin), soluble in ether and serves in Germany as the main source for making the resin which is termed there jalapin.

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[1] The British Pharmaceutical Codex restricts the name jalapin to the glucoside termed convolvulin above, but the latter term has found more general use in scientific literature and will be adhered to here.

Jalap Resin.

Jalap resin is prepared by digesting 100 parts of jalap with twice its weight of alcohol for 24 hours at a moderate temperature, transferring the mixture to a filter, allowing the liquid to pass and exhausting by percolation with more alcohol. 50 parts of distilled water are added to the filtrate, the alcohol removed by distillation and the residue cooled in an open dish. The resin is dried after washing with cold water.

According to the British Pharmacopœia it occurs in dark brown opaque fragments, translucent at the edges, brittle, readily reduced to a pale brown powder, and sweetish in odour. It dissolves readily in 90% alcohol, but is insoluble in oil of turpentine. To prove absence of scammony resin and resin of Tampico jalap, the powder should yield practically nothing to warm water and not more than 10% to ether. In the absence of guaiacum resin, a solution in alcohol is not coloured bluish-green by ferric chloride solution.

W. B. Cowie (*Pharm. J.*, 1908, **81**, 363) recommends the following tests: (1) Moisture. (2) Ash. (3) Solubility in ether.—1 grm. of the powdered resin is rubbed in a mortar with 10 c.c. of ether, the ether filtered through cotton wool, into a tared flask, and the operation repeated thrice. The ether is distilled and the residue weighed after drying at 110°. (4) Acid value.—1 grm. resin dissolved in 30 c.c., alcohol is used. (5) Saponification value.—1 hour's boiling with $N/2$ alcoholic potassium hydroxide and titration with $N/2$ hydrochloric acid. (6) Absence of colophony.—0.25 grm. resin in 5 c.c. acetic anhydride should give no purple colour with 2 drops of strong sulphuric acid. (7) Absence of guaiacum.—No greenish-blue colour with ferric chloride. (8) Resin yields no water soluble substance and is free from bitterness.

The ether soluble resin—convolvulin—has the acid value 2.8, saponification value 408, $[\alpha]_D - 39.5°$. Commercial "white jalap resin" gave moisture 3%, ash 0.02%, ether soluble 0.3%, acid value 2.8, saponification value 417. Two samples of brown jalap resin gave moisture 5.0 and 5.6%, ash 0.3%, ether soluble 10%, acid value 11.2 and 14, saponification value 333 and 338.

Scammony Root.

Scammony root is derived from *Convolvulus Scammonia*, a climbing plant indigenous to the Eastern Mediterranean. The roots are often

very large; small ones measure 6 to 12 in. long and 1 in. in diameter; large roots are 3 or 4 in. thick and 2 to 3 feet long. The transverse section shows a number of nearly circular bundles. The odour is characteristic like jalap and the taste acrid.

On an average the root contains 8% of glucoside resin (scammonin), starch, sugar and about 10% ash. The sugars comprise dextrose, sucrose, and small quantities of methyl pentose.

Scammony Resin.

The resin is prepared by extraction of the root with alcohol, removal of most of the alcohol by distillation and precipitation of the resin by pouring the residual liquid into ten times its volume of water. The resin is collected and washed with boiling water and dried.

It occurs in brownish translucent brittle pieces with a sweet odour and resinous fracture. It does not alone form an emulsion with water, a distinction from the scammony gum resin, is entirely soluble in ether and gives in alcoholic solution no blue colouration with ferric chloride or hydrogen peroxide, showing the absence of guaiacum resin.

Cowie (see page 132) examines scammony resin on similar lines to jalap. Two specimens of "white resin" were entirely soluble in ether and gave moisture 2.52 and 5.3%, ash 0.02%, acid value 2.8, saponification value 241, $[\alpha]_D - 25°$; it had m. p. 120°. Pure brown scammonin had 4.5 and 5.1% moisture, 0.15% ash, acid value 25.2 and 28, saponification value 263; it melts at 100°. The limit for acid value is 8.4 for white resins and 34 for brown. 95% of the brown resin should dissolve in ether.

Mexican scammony resin[1] has been examined by Cowie and Brander

[1]A more complete investigation of "Mexican Scammony Root" (*Ipomœa orizabensis*) is published by Power and Rogerson (*Trans.*, 1912, **101**, 1–26) of which the following is a summary.

The root was found to contain 14.55 % of resin, of which 71 % was soluble in ether. After treatment with animal charcoal the resin was obtained nearly colourless; it then melted at 125–130° and had $[\alpha]_D = -23.0°$.

For a complete examination of the root 48.76 kilograms was extracted with hot alcohol; a portion of the concentrated extract was used for determining the presence of sucrose and a small amount of the latter was isolated. Another portion of the extract was steam distilled when a very small amount of a pale yellow essential oil was obtained. From the portion of the extract which was soluble in water the following compounds were isolated:

(i) *Scopoletin*, $C_{10}H_8O_4$ (m. p. 203–204°), a small portion of which appeared to be present in the form of a glucoside.

(ii) 3:4-*Dihydroxycinnamic acid*, $C_9H_8O_4$ (m. p. 223–225°) from which the *methyl ester* (m. p. 158–160°) was prepared.

The aqueous liquid also contained a quantity of sugar which yielded *d*-phenylglucosazone (m.p. 205–206°).

The portion of the alcoholic extract which was soluble in water consisted of the above mentioned *resin*. The resin was extracted successively with various solvents.

I. *Petroleum Extract of the Resin.*—From this extract the following substances were obtained:

(i) *Hentriacontane*, $C_{31}H_{64}$;

(*Pharm. J.*, 1908, **81,** 366). About 70% is soluble in ether, the acid value is 8.4, saponification value 295 to 327.   4.8% is soluble in carbon bisulphide.

Taylor (*Amer. J. Pharm.*, 1909, **81,** 105) has published complete analyses of scammony resin from *Convolvulus scammonia* and of "Mexican Scammony" from *Ipomœa orizabensis*. The saponification value appears to afford a means of distinguishing the two resins.

Scammony.

Scammony is the gum resin obtained by incision from the living root. The emulsion is collected in shells and allowed to dry. It is usually imported in flattened cakes varying in colour from dark grey to brown or nearly black. Thin fragments are translucent and the fractured surface is glossy. It has a cheesy odour. It is easily reduced to an ash-grey powder and yields a milky emulsion when rubbed with water.

Pure scammony contains as much as 88 to 90% of ether soluble resin, the residue consisting of gum. Commercial qualities, known as virgin, usually vary from 75 to 85%, the official limit being 70% resin. It should not contain more than traces of starch and the ash must not exceed 3%.

Adulteration.—Scammony is frequently adulterated with starch, chalk, and earthy matter or with other resins. Foreign starches may be detected microscopically. Excess of ash will show inorganic matter.

(ii) *A phytosterol*, $C_{27}H_{46}O$;
(iii) *Cetyl alcohol*, $C_{16}H_{34}O$;
(iv) A mixture of fatty acids, consisting of palmitic, stearic, oleic and linolenic acids.
II. *Ethereal Extract of the Resin.*—The sp. rot. power of this extract was $[\alpha]D = -20.5°$.
After hydrolysis with barium hydroxide it gave:
(i) *Ipuranol*, $C_{23}H_{38}O_2(OH)_2$; (ii) *d-α-methylbutyric acid;* (iii) *tiglic acid;* and a product which on acid hydrolysis gave (iv) *jalapinolic acid*, $C_{15}H_{30}(OH)CO_2H$ (m. p. 67–68°; $[\alpha]D + 0.79°$), together with a little methyl jalapinolate and (v) a mixture of sugars consisting of dextrose and a *methylpentose*.   The latter yielded an osazone, m. p. 180–182°, and a *tetraacetyl derivative*, $C_6H_8O_5Ac_4$, which apparently is a new compound, m. p. 142–143°, $[\alpha]D = +21.64$.   The ethyl acetate extract of the product resulting from the alkaline hydrolysis of the ethereal extract gave on oxidation with nitric acid, a mixture of acids, consisting apparently of optically active valeric and hexoic acids, together with sebacic and *n*-nonanedicarboxylic acids.
III. *Chloroform Extract of the Resin.*—This was relatively small in amount and consisted of a dark resinous product.
IV. *Ethyl Acetate Extract of the Resin.*—The sp. rot. power of this extract was $[\alpha]D = -28.01$.   After hydrolysis with barium hydroxide it yielded products from which the same products were obtained as from the ethereal extract of the resin, with the exception of ipuranol.
V. *Alcoholic Extract of the Resin.*—This was a black amorphous product of a glucosidic nature, but which yielded nothing definite in hydrolysis.
It thus appears that the resin of *ipomœa orizabensis* is an exceedingly complex mixture the constituents of which are for the most part amorphous and not entirely glucosidic.   It follows therefore that the so-called "jalapin" which includes all the constituents which are soluble in ether, cannot be represented by any of the various formulæ hitherto suggested. The portion of this resin which is soluble in ether is not identical with the ether soluble portion of jalap resin as has previously been affirmed.

To detect resins the ether extract is dissolved in a hot solution of potassium hydroxide. Scammony resin is not precipitated on acidifying, whereas most foreign resins are.

Inferior scammony is usually tough, has a dull fracture and small splinters are opaque. A variety sold as skilleep usually consists of flour dough mixed with some of the gum resin. Evans, Sons, Lescher and Webb report on samples labelled "Aleppo" which contained 20 to 23.8% of ether soluble matter instead of about 50%. Nine samples of genuine Aleppo scammony had a range of 57.2 to 66.5% of resin.
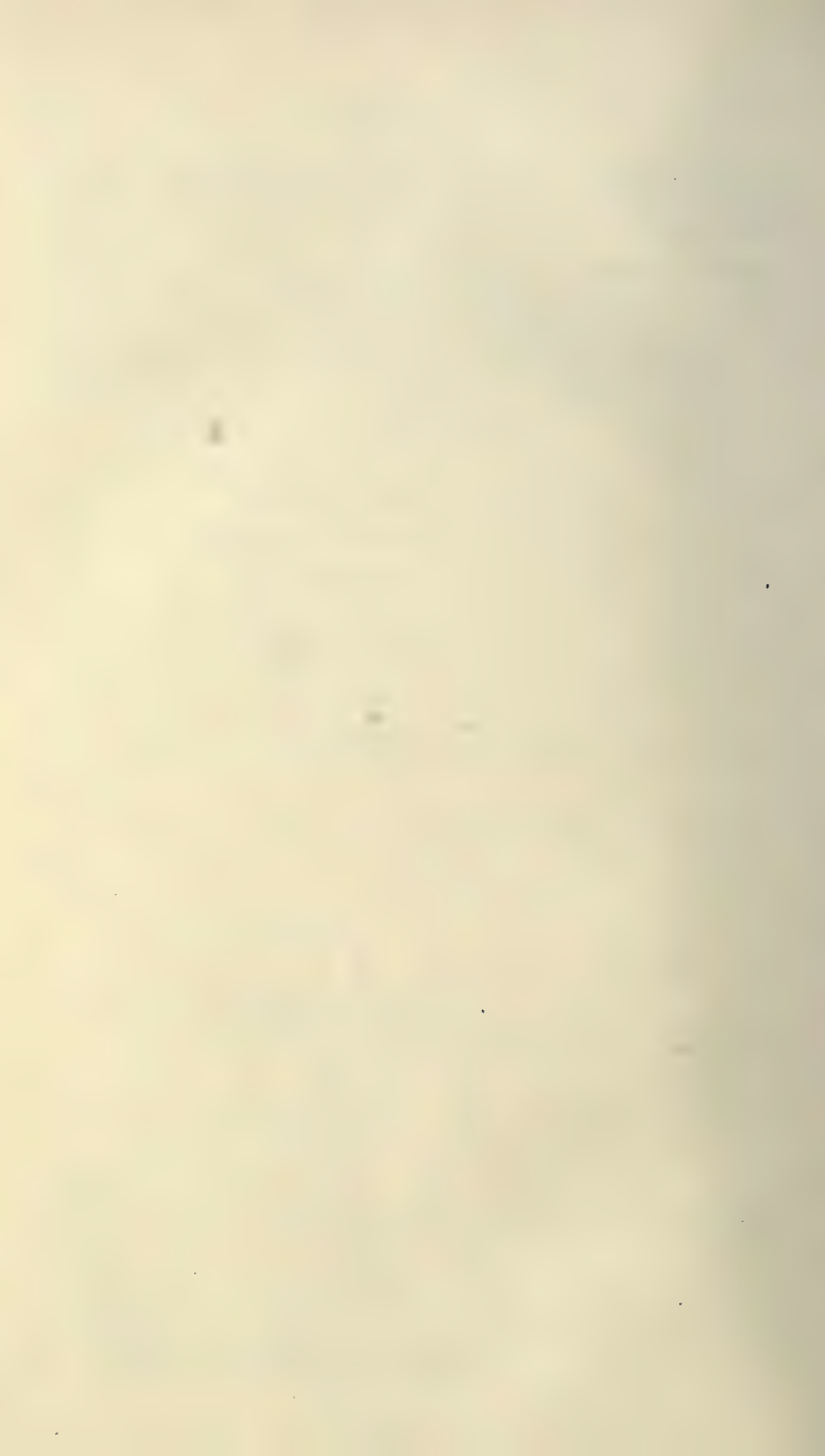
NON-GLUCOSIDAL BITTER PRINCIPLES

By G. C. JONES, F. I. C.

A considerable number of neutral and non-glucosidal bitter substances receive applications in medicine and as flavouring agents, etc. In many instances their chemistry has been very incompletely studied, while their feeble affinities render their available reactions few in number and wanting in precision.

The more important members of the group, *e. g.*, the aloïns, santonin, colocynthin, picrotoxin, hop-bitter, and quassiin are considered in the sequel.

Bach (*J. pr. Chem.*, 1874, **117**, 188; *Year-book Pharm.*, 1874, 293) has published some useful hints for the detection of certain bitter principles when occurring in medicine. He points out that the bitter principles of aloes, colocynth, worm-wood, and gentian are soluble in water, that those of agaric and scammony dissolve in ether, while jalap resin is insoluble in either solvent.

The scheme on next page for the detection of the above bitter principles in medicines, etc., is a tabulated form of Bach's method of procedure.

Aloes Bitters. Aloïns.

The article known in commerce as aloes or bitter aloes is the inspissated juice which has exuded or been pressed from the leaf of various species of *Aloe*.[1] It contains a number of closely allied purgative bitter principles generically called aloïns, which can be extracted from the corresponding aloes by treatment with water.

[1] Interesting information on the preparation of aloes from the juice of the plant is given in a paper by Holmes (*Pharm. J.*, 1892, **52**, 233).

Extract the substance or residue with alcohol, and evaporate the filtered liquid to dryness. Exhaust the dried and powdered residue with cold water, and filter.

Residue may contain resins of agaric, scammony, and jalap. Dry, and treat with ether.

Solution, concentrated at 100° if necessary, is cooled, treated with mercurous nitrate in excess, and rapidly filtered.

Residue consists of *jalap* resin, which gives with strong sulphuric acid a brown colouration, gradually becoming blood-red, and exhaling odour of *jalap*.

Solution. Evaporate and treat residue with warm solution of sodium carbonate.

Residue swells to yellow mass with nitric acid. Cold sulphuric acid dissolves it with orange-red colour, changing to cherry-red, *scammony*.

Solution. On acidifying, *agaric* resin is precipitated, insoluble in nitric acid, but dissolved by sulphuric acid with orange colour, becoming brown on heating, and decolourised by nitric acid, with separation of colourless flocks.

Precipitate is washed and dissolved in warm dilute nitric acid. *Colocynth* will be indicated by yellow coloration and separation of insoluble flocks; *wormwood* gives a brown solution, and few if any flocks. Ammonia is added in excess, and the filtered liquid evaporated to dryness. The residue is treated with warm acetic acid.

Residue contains *colocynth,* which gives a red colouration with strong sulphuric acid and a cherry-red with Fröhde's reagent.

Solution, if evaporated to dryness, yields a residue which, in presence of *wormwood,* is yellowish-brown, and gives with Fröhde's reagent a brown colouration, changing to green, and ultimately to violet.

Filtrate. Add ammonia in excess, filter, treat filtrate with barium acetate, and again filter.

Precipitate. Exhaust with alcohol, evaporate filtered solution, and test portions of residue with sulphuric acid, nitric acid, and alkali hydroxide, all of which give yellow colourations in presence of *gentian.*

Filtrate is orange-red in presence of aloes. Evaporate to dryness, exhaust with alcohol, again evaporate, treat residue with nitric acid, and evaporate to dryness. If *aloes* were present, the picric acid formed will give a brown-red colour when boiled with alkali hydroxide and a reducing agent, such as potassium cyanide.

Tilden (*Pharm. J.*, 1872, **31,** 845; 1875, **35,** 208) divided aloïns into two classes, nataloïns (characteristic of Natal aloes) and barbaloïns (characteristic of Barbadoes aloes).

1. *Nataloïns* are not reddened by treatment with nitric acid, even on heating; and do not form any definite chloro- or bromo-derivatives which yield only picric and oxalic acids as products of the oxidation.

2. *Barbaloïns* are reddened by nitric acid, and in addition to picric and oxalic acids yield aloëtic acid, $C_7H_2O_5N_2$, and chrysammic acid, $C_{14}H_4(O_2N)_4O_4$, as products of the treatment.

Shenstone (*Pharm. J.*, 1882, **42,** 461) further distinguishes *α-bar-baloïn* from Barbadoes aloes, which is reddened in the cold by nitric acid; and *β-barbaloïns* from Jafferabad, Socotrine, and Zanzibar aloes, which are not coloured in the cold by nitric acid of 1.42 sp. gr., though reddened on heating. The *β*-barbaloïns are, however, reddened by cold *fuming* nitric acid.

According to Sommaruga (*Pharm. J.*, 1874, **34,** 23) socotrine aloes contains a mixture of an aloïn of the composition $C_{15}H_{16}O_7$ with another of the composition $C_{17}H_{18}O_7$. Natal aloïn is said to contain $C_{25}H_{28}O_{11}$ or $C_{24}H_{26}O_{10}$.

Most discrepant statements are also recorded respecting the solubility, physiological activity, susceptibility to air and heat, and other characters of the aloïns (see Brown, *Pharm. J.*, 1887, **46,** 678). Thus, aloïn (source doubtful) is stated by various authorities to dissolve in 60, in 90, and in 500 parts of water; and to be insoluble, freely soluble, and soluble in 30 parts of alcohol. The British Pharmacopœia of 1898 describes aloïn from Barbadoes or Socotrine aloes as sparingly soluble in cold water, more so in 90% alcohol, freely soluble in hot water or alcohol, and nearly insoluble in ether.

According to the United States Pharmacopœia of 1907, aloïn from Curaçao aloes is soluble in about 120 parts of cold water, in 15 parts of alcohol, or in 55 parts of acetone. The United States Pharmacopœia requires aloïn to be almost free from emodin. "On shaking 1 gr. with 10 c.c. of benzene for 1 minute and filtering, the filtrate should not impart more than a faint pink colour to an equal volume of ammonia water (5%) when shaken with it."

Aloïn from Barbadoes aloes was obtained by Grönewold (*Arch. Pharm.*, 1890, **228,** 115; abst. *J. Chem. Soc.*, 1890, **58,** 639) in small, pale yellow, prismatic needles, melting at 147°. The air-dried crystals obtained from a concentrated alcoholic solution according to Gröne-

wold contain $C_{16}H_{16}O_7 + 3H_2O$. This has been confirmed by Tschirch and Pedersen (*Arch. Pharm.*, 1898, **236**, 200). $2H_2O$ are lost at 100°. The moist crystals readily become discoloured, especially if exposed to light. They dissolve very sparingly in cold water, but readily on boiling, the hot solution rapidly becoming brown. Barbaloïn is only slightly soluble in ether, chloroform, carbon disulphide, benzene or petroleum-spirit. Acetic acid dissolves it readily, and this solution is not affected by the air. A bromaloïn of the composition $C_{16}H_{13}O_7Br_3 + 4H_2O$ was described by Grönewold, who also obtained with difficulty a triacetyl-derivative, $C_{16}H_{13}O_7Ac_3 + 1/2H_2O$, in soft yellow needles, and a hexacetyl-derivative forming white, hard, columnar crystals. On oxidation with chromic acid mixture barbaloïn yields acetic acid, carbon dioxide, and alloxanthin, $C_{14}H_3(CH_3)-(OH)_4O_2$.

Grönewold regards the *aloïn from Curaçao aloes* as identical with that from Barbadoes aloes.

Aloïn from Natal aloes is said by Grönewold to contain $C_{24}H_{26}O_{10}$ or $C_{23}H_{23}(O.CH_3)O_9$, with a variable quantity of water of crystallisation. It forms bright yellow scales or large well-formed crystals, which soften in a capillary tube at 180° and melt and decompose at about 210°. These characters, together with its resistance to the action of alkalies and the presence of a methoxyl-group, distinguish nataloïn from barbaloïn. On treatment with chromic acid mixture, nataloïn yields acetic acid and carbon dioxide, quinol being probably formed in addition.

In alcoholic solution, the aloïns are neutral to litmus. Their aqueous solutions are coloured green or greenish-black by ferric chloride, and are gradually precipitated by a solution of basic lead acetate.

In acid or neutral solutions the aloïns are tolerably stable, but in presence of alkalies, in which they dissolve with orange colour, they readily undergo decomposition.

The action of dilute mineral acids on the aloïns has been studied with very discordant results. According to Czumpelik and Rochleder (1861), by treating aloïn with dilute sulphuric acid, dextrose and rottlerin are produced. Kossmann describes the products of the action as dextrose, aloëresic acid, $C_{30}H_{32}O_{14}$, aloëretic acid, $C_{30}H_{34}O_{15}$, and aloëretin, $C_{30}H_{18}O_{11}$. Tilden, however, denies the formation of dextrose. Hlasewetz states that paracoumaric acid is formed, and Rochleder and Czumpelik obtained the same substance by the action

of potassium hydroxide on aloïn. According to Tschirch and Pedersen when barbaloïn is fused with potassium hydroxide or heated with 10% sulphuric acid and steam blown through the mixture an intensely black powder, *alonigrin*, $C_{22}H_{18}O_8$, is formed, which is soluble in alkalies, but insoluble in ordinary solvents.

Great discrepancies occur in the recorded statements of the proportion of aloïn present in commercial aloes. The following method, described by Tilden, is regarded by H. C. Plenge (*Pharm. J.*, 1884, **44**, 330) as the best practicable plan of preparing aloïn on the small scale from most varieties of aloes: 25 grm. of the sample should be dissolved in boiling water, the liquid acidified with hydrochloric acid, and allowed to cool. It is then decanted from the precipitated resinous matter, evaporated to about 50 c.c., and set aside for two weeks for crystals to form. The liquid portion is then poured off, and the crystals pressed between folds of bibulous paper. The crude aloïn thus obtained is contaminated with a considerable quantity of resin, from which it is best purified by treating it with ethyl acetate, with occasional agitation, till the liquid acquires a brown colour, and the yellowish colour of the crystals can be distinguished. The liquid is then quickly and carefully poured off, and the crystals dried. Treated in this manner, Barbadoes aloes, for which the method is specially adapted, gave an average of 9% of aloïn; while Curaçao averaged 7.5, and Bonare 7%. Socotrine aloes yielded 3%, but on repeating the process on the same sample, no aloïn could be obtained. It was, however, isolated to the amount of 10% by digesting 2 parts of the aloes in 3 parts of alcohol for 24 hours, and then heating the liquid over a water-bath for 2 hours. After cooling, the liquid was poured off from the resin, filtered, and set aside in a loosely covered dish to crystallise. The crystals of aloïn were washed with a little alcohol, and dried.

Woodruff (*Pharm. J.*, 1889, **48**, 773), recommended the following process for the preparation of aloïn: 20 grm. of Barbadoes aloes were heated over the water-bath with about 40 c.c. of amyl alcohol, in a flask furnished with a reflux condenser. The liquid was decanted while hot into a beaker, where it solidified on cooling, and in 3 days formed a mass of which the major part consisted of impure crystals of aloïn. The mass was pressed and percolated with carbon disulphide to dissolve admixed resin, and the solvent then washed out with benzene. From the dried crude aloïn thus obtained a pure product was obtained by redissolving in cold water (hot water dissolves resinous matter),

filtering, and allowing the aloïn to crystallise spontaneously from the filtrate.

Other methods for the isolation and identification of the different aloïns are given under the heading **Valuation of Aloes.**

Serre (*Pharm. J.*, 1895, **54,** 839) gives the following data illustrating the quality of typical samples of commercial aloïn, which is now employed very extensively for the manufacture of pills.[1]

	Colour	M. p.	Resin	Ash
A. American.....	Bright pale yellow......	116°	. . . .	. . . .
B. American....	Brown................	140°	5.8%	1.4%
C. English.......	Greyish-yellow.........	145°	. . . .	4.7%
D. German......	Deep bright yellow.....	(softens only). 142°	. . . .	1.3%

Serre remarks that the ash of B was white, but the ash of C and D consisted chiefly of iron, to which impurity he attributes the dull colour of sample C. He adds that the best quantitative test for resin— the precipitation of an ammoniacal alcoholic solution with a large volume of ice-water—gave negative results in the cases of C and D, although the presence of resin could be detected qualitatively, and was indicated by the colour of the preparation. Serre recommends as a test for absolute freedom from resin that 1 grm. of the finely powdered aloïn should be shaken in a test-tube with 20 c.c. of water, and allowed to stand for 1 minute. The resultant solution should be perfectly clear. A was the only one of the above samples which stood this test. A melting-point of approximately 116° should, according to Serre, be insisted on. This condition is not consistent with recorded melting-points of pure aloïns.

Commercial Aloes vary in physical and chemical characters according to their origin. The chief varieties are those known as Barbadoes, Cape, Natal,[2] Hepatic, Socotrine, Zanzibar and Uganda[3] aloes.

[1] Some useful hints on the preparation of aloïn on a large scale have been published by Serre (*Pharm. J.*, 1895, 54, 839). He points out that complete combustibility and insolubility in alcohol are no indication of the absence of resin. In preparing aloïn it is usual to employ dark aloes as being of lower price, but such a practice is held by Serre to be objectionable. The selected aloes, which should be of a liver colour and clear fracture, are dissolved in water at a temperature not exceeding 40°, and, when solution appears complete, more water added as long as further precipitation of resin occurs. The liquid is then allowed to stand, and the bright solution concentrated *in vacuo* and set aside to crystallise. The mother-liquor is then drawn off, and the aloïn pressed and purified by suitable solvents and recrystallisation. The last traces of resin can only be removed from the aloïn by elaborate mechanical and chemical methods, impracticable on a small scale.
[2] Natal aloes have almost disappeared from commerce.
[3] It is said that "Uganda" aloes come not from Uganda, but from Mossel Bay in Cape Colony.

According to the British Pharmacopœia (1898) Barbadoes aloes is the juice that flows from the transversely cut leaves of *Aloe vera Linn.*, *Aloe chinensis*, *Baker*, and probably other species, evaporated to dryness. Imported from the West Indian Islands, and known in commerce as Barbadoes and Curaçao aloes. Socotrine aloes is described as the similar product from *Aloe Perryi*, *Baker*, and probably other species, imported principally by way of Bombay, and known in commerce as Socotrine and Zanzibar aloes.

The following tabular statement is compiled from the characters of Barbadoes and Socotrine aloes given in the British Pharmacopœia.

	Barbadoes Aloes	Socotrine Aloes
Colour	Yellowish or reddish-brown to chocolate-brown or almost black.	Brownish-yellow, becoming dark brown or nearly black on drying. Zanzibar variety. Liver-brown.
Fracture............	Either dull and waxy with opaque splinters, or smooth and glassy with transparent splinters.	Dull, waxy and uneven, with opaque splinters. Zanzibar variety. The same but smoother and more even.
Odour..............	Disagreeable................	Strong but not disagreeable.
Taste..............	Bitter and nauseous.........	Bitter and nauseous.
With nitric acid......	Crimson colour..............	Reddish or yellowish-brown.
Vapour of HNO₃ blown over powder previously mixed with H₂SO₄.	Only slight bluish-green. Not bright blue (absence of Natal aloes).	No blue colouration (absence of Barbadoes and Natal aloes).
Solubility in cold water.	Not less than 70%...........	About 50%.
In mixture of 2 vols. 90% alcohol with 1 vol. water.	Almost entirely soluble........	Almost entirely soluble.

The varieties which are opaque in small splinters exhibit, when examined under the microscope, numerous minute crystals embedded in a transparent mass.

The United States Pharmacopœia prescribes that aloes shall not lose more than 10% of its weight when dried at 100°, that it shall be almost completely soluble in 5 parts hot alcohol and that 5 grains should dissolve almost completely in 60 c.c. of boiling water and on cooling not more than 2 grains should separate.

Neither the British Pharmacopœia nor the United States Pharmacopœia prescribes a limit of ash. This varies in commercial samples from 1.7 to 4.1% and 3% has been suggested as a reasonable limit.

Besides Barbadoes and Socotrine aloes, aloes of other origin occur

in commerce and a number of tests, many of them originally put forward as tests for the detection of aloes but now known to be characteristic only of aloes of particular origin, are made use of to distinguish between the several commercial varieties.

Bornträger's test (*Zeitschr. anal. Chem.*, 1880, **19,** 165) consists in extracting the substance with alcohol, filtering, shaking the evaporated filtrate with benzene, removing the benzene layer and agitating it with ammonia, when the aqueous layer will, on standing, develop a more or less pink colour. The colour is not due to aloïn, but to alo-emodin (Tschirch and Pedersen, *Arch. Pharm.*, 1898, **236,** 200; Oesterle, *Arch. Pharm.*, 1899, **237,** 81). Though most, if not all, aloes give the reaction, it is not a safe test for aloes in admixture with other substances, but since aloes of different origin give rise to slightly different tints and intensity of colour, it is an aid in distinguishing these. Tschirch and Hoffbauer (*Schweiz. Woch. Chem. Pharm.*, 1905, **43,** 153) take 10 c.c. of a 1 in 1,000 aqueous solution for the test, shake for 1 minute with 10 c.c. of benzene, and pour the benzene into 5 c.c. of ammonia. A similar reaction is given by rhubarb and other drugs containing chrysophanic acid, hence the uselessness of the test for the detection of aloes. The difference in the intensity and shade of colour given by the several varieties of aloes is shown in the table of Cripps and Dymond on a succeeding page. It should be added that with some kinds of aloes a very considerable time (24 hours) is requisite for the development of the colour.

Klunge's test (*Arch. Pharm.*, 1883, **221,** 363) consists in the addition, to a highly dilute and therefore nearly colourless solution of aloes, of 1 drop of copper sulphate solution, followed by the addition of sodium chloride and alcohol. Léger (*J. Pharm. Chim.*, 1902, [vi], **15,** 335) operates on a 0.5% solution of aloes made by treating with hot water, cooling and filtering from deposited resin, and to 20 c.c. of the filtrate he adds 1 drop of a saturated solution of copper sulphate, and follows this by 1 grm. of salt and 10 c.c. of 90% alcohol. Tschirch and Hoffbauer (*loc. cit.*) add 1 drop of 5% copper sulphate to 10 c.c. of a 1 in 1,000 solution of the aloes, and follow this by a trace of salt and a few drops of spirit. On the addition of the copper sulphate, the original yellow colour of the solution is intensified. The addition of the salt, however, followed by a little alcohol or warming, changes this to a red colour, the intensity and permanence of which differs with the different varieties of aloes. The use of alcohol may be avoided

if the solution be warmed, but the addition of a considerable amount, as recommended by Léger, has the advantage that it dissolves the flocculent precipitate produced by the salt. The behaviour of the various sorts of aloes under this treatment is shown in the table of Cripps and Dymond, to which have been added two notes derived from Léger (*loc. cit.*).

Fluckiger's test is the British Pharmacopœia test with sulphuric acid and the vapour of nitric acid. Bainbridge and Morrow (*Pharm. J.*, 1890, **49**, 570) have applied this test to the juice of plants of known species growing at Kew and have shown that the deep blue colour given by **Natal** aloes is characteristic and is given by no other species. Every commercial specimen tested by these authors, however, gave some faint blue colour and the British Pharmacopœia still allows a bluish-green, but not bright blue in Barbadoes aloes.

Cripps and Dymond's table (page 146) shows the behaviour of the various sorts of aloes when subjected to the above three tests, as well as to a test devised by them. This test (*Pharm. J.*, 1885, **44**, 633) is conducted as follows: 1 grain of the solid substance to be tested (for this test is said to be applicable to the detection of aloes in admixture) is treated in a porcelain dish, or in a glass mortar standing on white paper, with 16 drops of strong sulphuric acid, and triturated until the whole is dissolved. Four drops of nitric acid of 1.42 sp. gr. are then added, and this is followed by 1 oz. of distilled water, when, in presence of aloes, a colour will be produced varying from deep orange to crimson, according to the kind of aloes employed. The result is confirmed by adding ammonia, when the colour is intensified, usually to a deep claret. The test not only allows of the detection of aloes, but gives a fair indication of the kind of aloes under examination.

Bainbridge and Morrow (*Pharm. J.*, 1890, **49**, 570) have observed that, on addition of nitric acid to Cape aloes, a reddish colour is at first produced, but this changes in the course of 5 minutes to a green, which is permanent for several hours. This reaction was always obtained with Cape aloes and was not simulated by any other variety. The only variety of Kew aloes which gave the reaction was prepared from a specimen of *Aloes Africana*. True Socotrine aloes was found to give no reaction with nitric acid, but every commercial specimen examined gave an evanescent crimson, and the British Pharmacopœia credits Socotrine aloes with the power of producing a reddish or yellowish-brown.

Variety of aloes	Bornträger's test	Klunge's test	Fluckiger's test	Cripps' and Dymond's test	
				On dilution	On adding ammonia
Barbadoes..............	Pale rose..............	Deep red[1].....	Faint blue.........	Crimson..........	Deep claret.
Natal..................	Very faint pink.........	Faint red.....	Deep blue.........	Deep crimson.....	Intense brownish-red.
Curaçoa................	Fine rose.............	Deep red[1].....	Faint blue.........	Crimson..........	Intense claret.
Hepatic................	Faint colour in 24 hours..	Nil.............	Nil...............	Orange-red.......	Claret.
Hepatic (Indian)........	Faint colour in 24 hours....	Nil.............	Nil...............	Orange-red.......	Pale claret.
Cape..................	Faint colour in 24 hours....	Nil[2].........	Nil...............	Orange-red.......	Pale claret.
Socotrine (true)........	Pale rose.............	Faint red[2].....	Very faint blue[3]....	Pale crimson.....	Deep claret.
Socotrine (commercial)..	Pale rose in 24 hours......	Nil.............	Nil...............	Orange-red.......	Claret.
Socotrine (commercial)..	Pale rose in 24 hours......			Orange-red.......	Claret.
Socotrine (commercial)..	Pale rose in 24 hours......			Orange-red.......	Claret.
Socotrine (Mocha or Zanzibar)......	Pale rose.............	Red........	Faint blue.........	Crimson..........	Deep claret.
Aloes juice (Natal)........	Pale brown-pink........			Crimson..........	Intense brown-red.

[1] Not disappearing within 12 hours (Léger: *J. Pharm. Chim.*, 1902, **15**, 335).
[2] According to Léger (*loc. cit.*) vinous red, fading within an hour to yellow, but Tschirch and Hoffbauer (*Schweis. Woch. Chem. Pharm.*, 1905, 43, 153) obtained negative results with Cape aloes.
[3] British Pharmacopoeia says no blue colour.

The so-called Uganda aloe has now been known in commerce for more than 10 years. It is said not to come from Uganda at all, but from Mossel Bay in Cape Colony, and its aloïn is identical with that of Cape aloes and it gives the same reactions, including the change from red to green after treatment with nitric acid (Bainbridge and Morrow test). It contains emodin and gives a faint colour within 24 hours under Bornträger's test. (*Cf.* Tschirch and Klaveness, *Arch. Pharm.*, 1901, **239,** 241).

Valuation of Aloes.

From time to time proposals have been made to value aloes from the proportion of crude aloïn which can be extracted under defined conditions. The method of Tilden, described under Aloïns, has been used in this way.

For the estimation of aloïn in aloes, Schäfer (*Pharm. Zeit.*, 1897, **42,** 95) has proposed the following method, based on the fact that aloïn, in ammoniacal solution, forms compounds with the alkaline earths, which are but slightly soluble, and from which the aloïn can be recovered on treatment with an acid:

50 grm. of aloes are treated with 300 c.c. of boiling water containing a few drops of hydrochloric acid, and when cold the solution is separated from the resin. 50 c.c. of 20% ammonia and 30 c.c. of 50% calcium chloride are then added and the whole well shaken. After 15 minutes, the precipitate is separated, and after being pressed is triturated in a mortar with a slight excess of hydrochloric acid. The aloïn and calcium chloride are dissolved in as little boiling water as possible and filtered. On cooling the aloïn separates in crystals. By his method, Schäfer found from 15 to 30% of aloïn in aloes of different origin.

Léger (*J. Pharm. Chim.*, 1902, [vi], **15,** 519) works on a 500-grm. sample, which he boils with a mixture of 1,800 c.c. of chloroform and 600 c.c. of methyl alcohol for 4 hours under a reflux condenser. After settling, the supernatant liquor is decanted and distilled, and the residue taken up with absolute alcohol, from which crystals of aloïns separate in 3 or 4 days. By this means, 5 to 6% of aloin was obtained from Cape aloes and from Barbadoes aloes, 10% from Curaçao aloes and as much as 20% from Jafferabad aloes. Socotrine aloes gave only 4%. The method, as described, cannot be considered as possess-

ing the attributes of a quantitative method, but Léger's object was mainly to get large quantities of the aloïns to serve for their separate detection and approximate estimation. Barbaloïn was identified by the characteristics of its chlor-acetyl derivative, which melts at 146.6°. and forms quadratic, yellow, anhydrous crystals only slightly soluble in hot alcohol, but very soluble in benzene. The aloïn which causes West Indian aloes to give a persistent red under Klunge's test is called by Léger *iso-barbaloïn.* Iso-barbaloïn can also be recognised by means of its bromine derivative, which is slightly soluble in cold alcohol, from which it crystallises in yellow needles. Socotrine aloes were found to consist almost exclusively of barbaloïn, with a minute quantity of iso-barbaloïn. Cape aloes were entirely free from iso-barbaloïn, while Curaçao aloes contained the two aloïns in approximately equal proportions. The aloïn of Jafferabad aloes is mainly iso-barbaloïn. Natal aloes contains neither barbaloïn nor iso-barbaloïn, but two distinct aloïns to which are due the characteristic reaction with sulphuric acid and the vapour of nitric acid.

Aloïn is not the only active constituent of aloes, alo-emodin having marked purgative properties; and Tschirch and Hoffbauer (*Schweiz. Woch. Chem. Pharm.*, 1905, **43,** 153, Abs. *Chem. Zeit. Rep.*, 1905, **29,** 106) propose to estimate the active constituents of aloes as follows: 5 grm. of the sample is digested in a 50 c.c. flask with 5 c.c. of methyl alcohol for 2 hours. The liquid is then warmed to 50 to 60°, 30 c.c. chloroform is added gradually with continuous stirring and the mixture allowed to stand for 30 minutes. The yellow-coloured chloroform solution is then filtered from the separated resin, caught in a tared flask and the chloroform distilled off and used again to extract the aloïn remaining with the resin, etc., in the first flask. The second chloroform extract is filtered into the tared flask, and the chloroform distilled off and again used to extract the residue in the first flask, this procedure being repeated four times in all. When the chloroform has been distilled off for the last time, not less than 4 grm. of extractive matter should be found in the tared flask. The residue in the original flask is regarded as valueless resin and the authors found 63% of such "resin" in Socotrine aloes, 33% as the average for Curaçao and only 13 to 19% in Cape aloes, which they prefer.[1]

L. v. Itallie (*Pharm. Weekblad*, 1905, **42,** 553; Abs. *Pharm. J.*, 1905,

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[1] Other continental authorities express a preference for Cape aloes, but in Great Britain their chief use is said to be in veterinary practice.

75, 554) considers the above method open to objection on the ground that much is left behind and counted as resin which is not resin at all, but other matters protected from the solvent action of the chloroform by a coating of resin. He recommends the following modification: 5 grm. of the powdered sample is warmed with 5 c.c. of methyl alcohol in a 50 c.c. flask until a homogeneous liquid is obtained. 30 c.c. of chloroform is then added and the mixture violently and continuously shaken for 5 minutes. By this time most of the separated resin will have adhered to the sides of the flask. After standing till clear, the liquid is decanted off. The residue is again dissolved in methyl alcohol and the resin again precipitated by means of chloroform, and this treatment is repeated once more. In this way v. Itallie found 18 to 43% of resin in Cape aloes, and only 11 to 21% in Curaçao aloes. The same author attempted the estimation of aloïn by precipitation as tribromoaloïn. With pure aloïn the method was successful, but, applied to aloes from the Cape and Curaçao, it indicated over 70% of aloïn, which v. Itallie considers to be much too high.

Detection of Aloes.

The aqueous solution of aloes is coloured dark brown by alkalies, and olive-green or greenish-black by ferric chloride.

According to W. Lenz, if aloes be extracted with amyl alcohol, the solution evaporated, the residue treated with nitric acid and again evaporated, and this second residue boiled with potassium hydroxide and potassium cyanide, a blood-red colouration will be obtained. This test is evidently based on the formation of picric acid by the action of nitric acid on aloin and its subsequent reduction to picramic acid by treatment with potassium cyanide.

Lenz also finds that useful reactions for aloes are obtained by treating an aqueous solution of the amyl alcohol extract with basic lead acetate, mercurous nitrate, tannin, and brominated potassium bromide. The extract reduces gold chloride and alkaline cupric solutions.

Weak (0.1% to 1%) aqueous solutions of aloes develop a green fluorescence when 5% of their weight of powdered borax is added to them.

For the detection of aloes in pharmaceutical preparations, Léger (*J. Pharm. Chim.*, 1902, 15, 335) treats 1 grm. of the sample with 100 c.c. of hot water, cools rapidly, and filters from deposited resin, the

filtration being accelerated by the addition of a little powdered talc.
A few decigrams of sodium peroxide are introduced little by little into
20 c.c. of the filtrate, previously heated to 90°. An immediate evolution
of oxygen occurs, and the liquid becomes brown, subsequently chang-
ing to bright cherry-red on further addition of peroxide, if aloes be pres-
ent. The reaction is said to be due to the alo-emodin of Tschirch
and Oesterle (*Arch. Pharm.*, 1898, **236**, 205; 1899, **237**, 81) and
like Borntrager's reaction is given by other drugs, such as rhubarb,
which contain chrysophanic acid (dihydroxymethylanthraquinone),
an emodin (trihydroxymethylanthraquinone) or rhein (tetrahydroxy-
methylanthraquinone), which dissolve in sufficient quantity to
falsify the results. The presence of hydroxymethylanthraquinones
can be detected by adding a small quantity of sodium hydroxide
solution, with which they give an immediate red colouration, while the
solution of pure aloes remains yellow. If hydroxymethylanthra-
quinones be detected in this way, they may be removed from the bulk
of the solution to be tested in either of two ways. Either they may be
precipitated by addition of a slight excess of basic lead acetate, and
this method is perferred by Léger, or they may be removed as follows:
To 50 c.c. of the original 1% solution to be tested, 20 c.c. of 5% alum
solution is added, and this is followed by excess of ammonia and
finally by acetic acid until the liquid is just acid. The mixture is then
filtered, and 20 c.c. of the clear filtrate tested with sodium peroxide as
described. If aloes be present a distinct reaction will be given,
while rhubarb extract treated in the same way gives only a faint peach
colouration.

Cripps and Dymond have applied their test (already described among
tests for distinguishing varieties of aloes) to various complex mixtures,
and they found that aloes could always be detected when present and, of
the numerous pharmaceutical preparations tried, no other substance
gave a similar reaction except senna, and substances, such as rhubarb,
which contained chrysophanic acid. But a nearly colourless aqueous
solution of aloes is not changed by ammonia, whereas with rhubarb
and other substances containing chrysophanic acid a pink colour is
developed. An ethyl acetate extract of rhubarb is coloured deep red
on treatment with strong sulphuric acid, as is a similar extract from
aloes. But on subsequently adding nitric acid the colour due to aloes
is intensified, whereas that produced by chrysophanic acid is immedi-
ately destroyed. This behaviour allows of the detection of aloes in

presence of rhubarb, etc., while the pink colour produced in an aqueous extract on addition of ammonia permits of the converse being effected.

For the estimation of aloes in mixtures, H. Hager evaporates the liquid to dryness, and macerates the cooled and pulverised residue with a mixture of 2 vols. of chloroform, 3 of benzol, and 1 of absolute alcohol. This dissolves the resins of jalap, scammony, myrrh, senna, guaiacum, etc. The residue, which contains the aloes inact, is treated at 50° with 80% alcohol. The solution is evaporated to dryness in a weighed dish, and the residue treated with 12 to 15 c.c. of a 2% solution of ammonia for every grm. of residue. To the filtered liquid an excess of lead acetate is added, and a few drops of ammonia to ensure an alkaline reaction. The precipitate, which contains all the aloes, is washed with water and mixed with ammonium sulphate. The mixture is then exhausted with 80% alcohol, and the weight of aloes ascertained from that of the residue left on evaporating the filtered alcoholic solution.

For the detection of aloes in animal matters, such as fæces, J. Dietrich (*Dorpat Thesis*, 1885; abst. *Analyst*, 1885, **10**, 186) digests the substance with water acidified with sulphuric acid, then macerates for 12 hours with 3 vols. of strong alcohol, concentrates the filtered liquid, and agitates the residue successively with petroleum-spirit and amyl alcohol. On evaporating the latter liquid, the aloïn is obtained in a state fit for the application of the ordinary tests. Dietrich treats it with nitric acid, evaporates at 100°, dissolves the residue in alcohol, and treats the deep red solution with a drop of an alcoholic solution of potassium cyanide, which produces a rose colouration in presence of aloïn. Dietrich is of opinion that on taking aloïn or aloes the greater portion is excreted with the fæces; a small portion only is absorbed and passes mostly through the kidneys, while the remainder enters the liver, and with the bile is conveyed back into the intestines.

Artemisia Bitters.

Various species of *Artemisia* contain non-glucosidal bitter principles, of which santonin is the most important. Absinthiin is a bitter principle contained in wormwood.

Santonin, $C_{15}H_{18}O_3$, is commonly obtained from Santonica, also called "worm-seeds" (*Flores cinæ*), which consist of the unexpanded

flower-heads of *Artemisia maritima, A. cina*, or of closely allied varie-
ties, which contain from 1 1/2 to 2% of the bitter principle.[1]

Santonin is the anhydride or lactone of santoninic acid, $C_{15}H_{20}O_4$, a
derivative of naphthalene.

A very large number of derivatives of santonin have been studied,
especially by Cannizzaro, Gucci, Grassi-Cristaldi, Andreocci, Frances-
coni, Montemartini and Wedekind. For a convenient summary of
this work see the Monograph "Die Santoningruppe" by E. Wedekind
in Ahren's "Sammlung chemischer und chemisch-technischer Vor-
träge," 1903. Several subsequent papers have appeared, more particu-
larly by Wedekind and Francesconi and Cusmano. The chemistry of
this group is not yet fully worked out, but santonin is probably
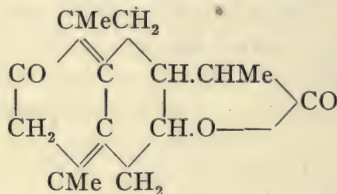
Santonin crystallises in flattened columns, in feathery radiating
groups, or in pearly plates having a slightly bitter taste. It has a
sp. gr. 1.1866, melts at 171 to 172° (Menge, *U. S. Hygienic Lab.
Bulletin*, **70**), and when heated cautiously may be sublimed unchanged.
When more strongly heated santonin becomes reddish-brown, evolves

[1] For the preparation of santonin from worm-seeds, the volatile oil is first extracted by
petroleum-spirit, and 200 grm. of the residue then boiled with 70 grm. of slaked lime, 400
c.c. of water, and 400 c.c. of rectified spirit, this treatment being twice repeated. The liquid
is filtered, evaporated to about 500 c.c., and a little hydrochloric acid added. The greenish
resin thereby separated is filtered off, and the filtrate treated with a slight excess of hydro-
chloric acid. This liberates santoninic acid, which soon decomposes with formation of santo-
nin, which crystallises out. The product is purified by washing with cold water, solution
in alcohol, decolourisation by animal charcoal, and recrystallisation.

The manufacture of santonin on a large scale is carried out at Tschimkent, which is
conveniently situated to the principal source of the plant on the Kirghiz steppes. The
worm-seeds are ground with lime and water, hot alcohol added to the cooled mixture, the
alcohol distilled off, and the residual liquid neutralised with hydrochloric ac d. The crude
santonin which crystallises out after a few days is washed with cold water. During the
process of extraction a quantity of resinous substance is formed, which on treatment with
a warm solution of sodium carbonate yields a large quantity of santonin. The animal
charcoal which is employed to decolourise the alcoholic solution absorbs a large quantity
of santonin. The yield of pure santonin obtained amounts to 1.8 to 2.0% of the plant
originally taken. A. Busch recommends the separation of the accompanying resin from
crude santonin by the addition of lead acetate to the alcoholic solution (*J. prakt. Chem.*,
1887, **35**, 322; abst. *J. Soc. Chem. Ind.*, 1887, **6**, 559). According to a modified process, the
worm-seed is treated with milk of lime, the extract precipitated by sodium carbonate, and
the filtered liquid warmed to 70° and decomposed by sulphuric acid. On cooling, santonin
separates in large crystals.

To test the solutions, etc., obtained in the process of extraction for santonin, J. Kossa-
nowski precipitates any colouring matter with basic lead acetate, and gently heats a few
drops of the filtrate in a porcelain crucible. Concentrated sulphuric acid is then added,
which on further heating will produce a violet colouration, the intensity of which is an in-
dication of the amount of santonin present (*Dingl. polyt. Jour.*, 1888, **268**, 42; abst. *J. Soc.
Chem. Ind.*, 1888, **7**, 422, 458).

white fumes, and on cooling sets to a clear brown vitreous mass, which is reddened on treatment with a little dry alkali hydroxide or slaked lime.

On exposure to light, especially to direct sun-light, santonin acquires a yellow colour. The hot alcoholic solution of this altered substance is yellow, but deposits crystals of colourless santonin on cooling.

Santonin is very sparingly soluble in cold water (1:5,000 at 15°), but dissolves in 250 parts of boiling water. It is soluble in 40 parts of cold rectified spirit, and in 3 parts at the boiling-point; in 160 parts of cold or in 42 of boiling ether, and in 4 parts of chloroform. The solution of santonin in boiling water is scarcely perceptibly bitter, but the cold alcoholic solution has an extremely bitter taste, and is lævorotatory. For an alcoholic solution $[\alpha]_D = -173.8$; in chloroform $[\alpha]_D = -171.4$. The concentration has practically no effect on the specific rotation. Solutions of santonin do not redden litmus, but the solid substance dissolves readily in alkaline liquids to form santoninates. On adding excess of hydrochloric acid to the alkaline solution, and immediately shaking the milky liquid with ether, santoninic acid is extracted.

Santoninic acid, $C_{15}H_{20}O_4$, rapidly separates from its ethereal solution in granules, and by recrystallisation from alcohol is obtained in fine rhombic crystals. It does not become yellow on exposure to light, and is very sparingly soluble in cold but more readily in hot water. The solution has an acid reaction, and is lævorotatory ($[\alpha]_D = -25.8$ in 97% alcohol, $v = 1-3$). When heated for some time at 120°- santoninic acid decomposes into santonin and water, and the same reaction occurs more readily on addition of a mineral acid to its solution, especially on warming.

Sodium santoninate, $C_{15}H_{19}O_4Na + 3H_2O$, forms colourless rhombic crystals, soluble in 3 parts of cold water, and also soluble in alcohol.

Calcium santoninate, formed in the extraction of santonin from worm-seed, crystallises in colourless silky needles, soluble in water and in dilute spirit, but almost wholly insoluble in absolute alcohol. The salt is not decomposed by carbon dioxide, but stronger acids cause a separation of santonin.

Santoninates of the heavy metals can be obtained by precipitation. When boiled with water such a santoninate is decomposed into pure santonin and the corresponding metallic oxide.

On boiling santonin for 12 hours with strong baryta-water, acidifying

the liquid with hydrochloric acid, and shaking with ether, santonic acid, $C_{15}H_{20}O_4$, is extracted. This substance is more stable than its isomer, santoninic acid. It forms rhombic crystals which melt at 161 to 163°, is unacted on by light, and dissolves sparingly in water but readily in alcohol to form lævorotatory solutions ($[\alpha]_D = -74°$ in chloroform).

When treated with excess of mineral acids, santonin forms santonin resin. This product is obtained most readily by heating santonin with concentrated hydrochloric acid under pressure. It is formed during the manufacture of santonin, and appears to be a mixture of products of its decomposition.

When solid santonin is agitated with a 5% solution of potassium hydroxide in alcohol, it dissolves with transitory carmine-red colouration. The test may be modified by moistening a mixture of equal parts of slaked lime and sodium carbonate with alcohol, and adding santonin, when a fugitive purple-red colouration is developed.

Pain (*Pharm. J.*, 1901, **67,** 131) has improved on this test as follows: A few crystals of santonin (1 mg. serves) are warmed with 2 to 3 c.c. of ethyl nitrite solution (British Pharmacopœia). Unlike aloin, resorcinol and many other substances, santonin gives no red colour with ethyl nitrite alone, but, on addition of a few drops of potassium hydroxide solution, a fine rose-red colour is produced. Thymol gives a dark yellow colour if present in a solution tested in this manner.

According to A. Busch, dextrose is a product of the action of dilute sulphuric acid on santonin.

If santonin be dissolved in slightly diluted sulphuric acid, the solution warmed on the water-bath, and a few drops of ferric chloride added, p ring of fine red colour, changing to purple, is developed round each droa of the reagent, and on continuing the heating the purple colour changes to brown. T. Salzer applies the test by treating 0.010 grm. of santonin with 1 c.c. of strong sulphuric acid and 1 c.c. of water. This mixture acquires a yellow colour, and on then adding ferric chloride and warming the violet colouration is produced.

Reichard (*Pharm. Zeit.*, 1907, **52,** 115) gives the following reactions of santonin. A mixture of santonin with ammoniated mercury or with mercurous nitrate is coloured black on adding sulphuric acid. A mixture of santonin with cupric sulphate or chloride when treated with sulphuric acid gives a fine blue colour. Bismuth subnitrate and santonin also give a deep blue colour when touched with sulphuric

acid. Diphenylamine, santonin and sulphuric acid do not interact when cold, but on warming the mixture becomes intensely red; the colour changes to yellow after about 12 hours and afterward becomes dark green. Cold sulphuric acid alone does not give a colouration with santonin, but on warming an intense blue colour is developed which on further warming becomes yellow.

When santonin is treated with dilute phosphoric acid, and the solution evaporated at 100°, a yellow colouration is produced, subsequently changing to purple-red.

If a solution of santonin in strong sulphuric acid be warmed on the water-bath for a few hours, and then diluted with water, iso-santonin, $C_{15}H_{18}O_3$, isomeric with santonin itself, is precipitated. When recrystallised from boiling alcohol this melts at 138°. It also differs from santonin in being unaltered by exposure to light, and in giving no red colouration with sulphuric or phosphoric acid.

For the *estimation* of santonin in santonica, Katz (*Arch. Pharm.*, 1899, **237**, 245) extracts 10 grm. of the coarsely powdered worm-seeds with ether in a Soxhlet extractor for 2 hours. After distilling off the ether, the residual, dark-green, resinous mass is boiled under a reflux condenser with 100 c.c. of 5% barium hydroxide for 15 to 30 minutes. When cool, the liquid is saturated with carbon dioxide until it has an acid reaction, when it is filtered with the use of a pump, and the carbonate washed twice with 20 c.c. of water. The pale yellow filtrate is concentrated on the water-bath to about 20 c.c., mixed with 10 c.c. of dilute hydrochloric acid (sp. gr. 1.06) and heated for not more than 2 minutes longer. The liquid is brought into a separator, the crystals of santonin in the basin are washed into the separator by means of 20 c.c. of chloroform, and, after shaking, the chloroform layer is run off and filtered through a paper previously moistened with chloroform. The basin in which the aqueous solution was concentrated is washed with 20 c.c. chloroform, which is then transferred to the separator, shaken with the aqueous contents of this and also run through the filter. This washing process is repeated once more. The chloroform is distilled off, and the residue boiled with 50 c.c. of 15% alcohol. The flask is covered with a watch glass and left at room temperature for 24 hours. By this time most of the santonin will have crystallised out, as it is but sparingly soluble in cold dilute alcohol. The solubility is not negligible, however, and the flask is next weighed and, for each 10 grm. contents, 6 mgm. is to be added to the weight of santonin

found later. The solution is next filtered through a tared 9 cm. filter, disregarding any cloudiness of the filtrate due to traces of resin, and the flask and filter are washed twice with 10 c.c. of 15% alcohol. This quantity of cold, dilute alcohol does not dissolve any appreciable amount of santonin during the short time that it is in contact with the relatively large crystals, and no correction is made for it. Finally the filter is folded and placed in the tared flask, which is then dried and weighed. The final weight, less that of the flask and filter, plus the correction for solubility, gives the weight of santonin in the 10 grm. sample taken.

When taken internally, santonin produces a remarkable effect on the vision, all objects appearing at first of a bluish tint, but subsequently yellow or greenish-yellow.[1] The taste and smell are also affected in some instances. In larger doses, santonin produces marked poisonous effects, the principal symptoms being headache, giddiness, shivering, stertorous breathing, followed by tetanus, diminished action of the heart, convulsions, and finally death by asphyxia. The pupils are dilated, and vomiting occurs in some cases. The *postmortem* appearances are not characteristic.

For the detection of santonin in urine, Daclin (*J. Pharm. Chim.*, 1897, **6**, 534) treats 30 to 40 c.c. with lead acetate and then with solid sodium sulphate and filters. The filtrate is divided into two portions, each of which is evaporated in a porcelain basin. The residue in one is gently warmed with 1 or 2 drops of sulphuric acid, when, if santonin is present, an immediate violet colouration appears. The other residue is tested with alcoholic potassium hydroxide. Or urine may be extracted with chloroform, the latter evaporated, and the residue tested as described.

In consequence of the occurrence of several accidents due to the contamination of santonin with strychnine, new tests have been introduced into the German and United States Pharmacopœias with a view to excluding any preparation containing alkaloids. The United States Pharmacopœia (1907) test is as follows: If 2 grm. of santonin be boiled with 80 c.c. of water and 5 c.c. of diluted sulphuric acid, and the liquid, after frequent shaking, be allowed to become cold and then filtered, mercuric potassium iodide or iodine should produce no cloudi-

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[1] This curious and characteristic effect is apparently due to a direct action of santonin on the nervous elements of the retina, rendering the eye less sensitive to the rays of high refrangibility (small wave-length). The eye thus becomes colour-blind to the violet end of the spectrum. Hence only the less refrangible rays produce a visual impression, and all objects appear yellow.

ness in 10 c.c. of the filtrate, mixed with 10 c.c. of distilled water, even after standing for 3 hours.

Absinthiin, $C_{15}H_{20}O_4$, is a glucosidal bitter principle existing in wormwood, *Artemisia absinthium*, wherein it is associated with an essential oil, to which is ascribable the toxic action sometimes observed as the result of absinthe drinking. The tonic effects of wormwood are due to the absinthiin.

For the preparation of absinthiin, the plant is exhausted with ether, the ether distilled off, and the residue taken up with water. The filtered liquid is shaken with hydrated alumina, again filtered, and evaporated *in vacuo;* or shaken with ether and the ether evaporated. Absinthiin thus obtained is described by O. Senger (*Arch. Pharm.*, 1892, **230,** 94) as a yellowish, vitreous, intensely bitter substance, melting at 65°. It is soluble in water, alcohol, and ether. When boiled with dilute sulphuric acid, absinthiin yields dextrose, a volatile product which appears to be an ethereal oil, and an amorphous resinous compound of the formula $C_{21}H_{26}O_6$, which is apparently a hydroxy-acid of the aromatic series. It yields phloroglucinol by the action of alkali hydroxides, while formic, acetic, and propionic acids are among the products of its oxidation by chromic acid mixture. When treated with concentrated nitric acid, absinthiin yields oxalic and picric acids.

Colocynth Bitter.

The fruit of the colocynth or bitter apple, *Citrullus colocynthis*, was until very recently supposed to contain a neutral bitter principle called colocynthin. This was extracted from bitter apples by exhausting the fruit with alcohol, evaporating the tincture to dryness, taking up the residue with water, precipitating the liquid with lead acetate, removing the excess of lead from the filtrate, and precipitating the colocynth by tannin. The compound with tannin was then decomposed by treatment with lead carbonate, and the colocynthin dissolved out by boiling alcohol.

Colocynthin was described as forming a yellowish powder or microscopic crystals, intensely bitter, and poisonous; readily soluble in water and in boiling alcohol to form solutions neutral to litmus. The alcoholic solution was precipitated on addition of ether.

According to Walz, colocynthin was decomposed by treatment with acids with formation of dextrose and *colocyntheïn*. G. Henke, on the other hand, stated that colocynthin was unaffected by dilute acids.

Concentrated sulphuric acid dissolves "colocynthin" with orange-red colour, carbonisation occurring on heating. In concentrated hydrochloric acid "colocynthin" dissolves with colouration, and on boiling a dark green greasy substance is precipitated, which, after drying over sulphuric acid, is only partially soluble in ether. The mother-liquor from which this precipitate is obtained reduces Fehling's solution.

With sulphomolybdic acid, "colocynthin" is said by Johannson to give a cherry-red colouration; with sulphovanadic acid, a blood-red colour changing to blue at the edges; and a yellow colouration with alcohol and sulphuric acid (distinction from solanine and solanidine). Moistened with phenol and a drop of sulphuric acid, "colocynthin" gives a blood-red colouration changing to orange.

"*Colocynthein*" is not so soluble in water as "colocynthin," but is dissolved by ether and benzene, and is sparingly soluble in petroleum-spirit. It may be extracted from acidified aqueous liquids by agitation with benzene, and the "colocynthin" can then be removed by ethyl acetate.

According to Power and Moore (*Trans.*, 1910, **97**, 99) "colocynthin" and "colocynthein" are not definite individuals. On extracting the pulp of *Citrullus Colocynthis* (Schrader) with alcohol and subjecting the resulting extract to distillation with steam, a very small amount of a pale yellow essential oil was obtained. From the portion of the extract which was soluble in water the following substances were isolated:

(1) A new dihydric alcohol, $C_{22}H_{36}O_2(OH)_2$, (m. p. 285 to 290°) termed *citrullol*, which is apparently a lower homologue of ipuranol and yields a diacetyl derivative, m. p. 167°. (2) An amorphous, alkaloidal principle, which is a weak base and from which no crystalline derivative could be prepared; it possesses an extremely bitter taste and represents one of the purgative principles of the fruit. The aqueous liquid from which the above mentioned compounds were isolated contained, furthermore, a quantity of inorganic salts, a little sugar and a very small amount of an amorphous glucoside substance.

The portion of the alcoholic extract, which was insoluble in water, consisted chiefly of resinous material, from which, however, a quantity of α-*elaterin* (m. p. 232; $[\alpha]_D - 68.9$) was isolated (compare *Trans.*, 1909, **95**, 1989). After the separation of the latter substance the resin was extracted with various solvents, which yielded a small amount of *hentriacontane*, $C_{31}H_{64}$ (m. p. 68); a *phytosterol*, $C_{27}H_{46}O$ (m. p. 160

to 162°, optically inactive); a mixture of fatty acids and a further quantity of α-elaterin, together with a little of the above-described alkaloidal principle. None of the extracts from the resin were glucosidic. The ether and chloroform extracts possessed marked purgative properties, even after the removal of the active alkaloidal principle.

The seeds of the colocynth, which represented 75.5% of the entire peeled fruit, were found to contain traces of an alkaloidal principle, a small amount ·of an enzyme which hydrolyses β-glucosides and a quantity of fatty oil corresponding to 12.72% of the weight of the seed. The constants of the fatty oil and of the total fatty acids obtained therefrom were determined and from the oil a small amount of a phytosterol $C_{20}H_{34}O$, was isolated, which melted at 158 to 160° and had $[\alpha]_D + 8.1°$.

The amount of glucosidic substance in the fruit is extremely small. The purgative action is due to at least two principles, one of which is alkaloidal, although a very weak base and incapable apparently of forming any crystalline salts, while the other source of activity is represented by some non-basic principle or principles contained in both the ether and chloroform extracts of the resin. All attempts to obtain the last mentioned active principle in a more definite form were, however, unsuccessful. No evidence could be obtained of the presence of β-elaterin, which constitutes the physiologically active constituent of *Ecballium elaterium*.

Colocynth pulp is obtained from at least three continents in the old world, but two principal varieties are recognised, Turkey colocynth and Spanish colocynth. Of these the former is whiter in colour and contains a larger proportion of pulp. The fruit is collected when ripe, freed from rind and dried. Occasionally it is imported unpeeled.

The colocynth pulp of commerce is usually in the form of balls about 2 in. in diameter, often broken. Each fruit consists of a yellowish-white pith-like pulp, in which a large number of hard brownish-white seeds are contained, arranged in 6 vertical rows. The seeds, which are oil-bearing, make up 75% of the weight of the drug as imported.

The British Pharmacopœia (1898) defines colocynth as "the fruit of *Citrullus colocynthis*, freed from seeds," the United States Pharmacopœia (1907) as "the peeled fruit of *C. c.* . . . The seeds should be separated and rejected."

The British Pharmacopœia requires colocynth pulp to be free from starch, to contain not more than traces of fixed oil removable by ether

(absence of seeds) and to contain at least 9% of ash (freedom from seeds which contain but 2 to 3% of ash).

The pharmacist, therefore, is required to separate the seeds, which is done by breaking the fruits open and sifting.

Chattaway and Moor (*Analyst*, 1903, **28**, 205) are in favour of reducing the standard of ash to 8%, and Umney (*Pharm. J.*, 1903, **71**, 879) says the ash in genuine samples may vary from 7.2 to 13.5%. Umney thinks a microscopical examination (*cf.* British Pharmaceutical Codex) and ascertaining that the sample is free from fixed oil are the best means for detecting adulteration.

Dowzard (*Pharm. J.*, 1903, **71**, 400) has pointed out that pure colocynth pulp yields about 3% of soluble matter when extracted with ether, and that this can scarcely be called a trace. The greater part of the ethereal extract, however, is "colocynthin," not oil, and Dowzard recommends the use of petroleum spirit in which "colocynthin" is insoluble. When using this solvent, Dowzard in no case found more than 1.3% of oil in pure pulp and he suggests that 2% might be fixed as the maximum amount permissible.

Bitters of Cocculus Indicus.

The seeds of *Anamirta paniculata* or *Cocculus Indicus* contain several bitter principles, of which picrotoxin is the most characteristic.

Picrotoxin, according to Paternò and Oglialoro, confirmed by Schmidt and Löwenhardt, contains $C_{30}H_{34}O_{13}$. For the extraction of picrotoxin, Barth and Kretschy exhaust the cocculus berries with boiling alcohol or petroleum-spirit, evaporate the filtered solution, and extract the fatty residue with water. The aqueous extract is precipitated with neutral lead acetate, filtered, the filtrate treated with hydrogen sulphide, and the filtered liquid concentrated to a small bulk, when crystals of impure picrotoxin separate on cooling.

Barth and Kretschy believed the product thus obtained to be a mixture of picrotoxin, picrotin, and anamirtin, which compounds they obtained from it by repeated crystallisations from benzene or water;[1] but Paternò and Oglialoro regard all three substances as

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[1] **Picrotin,** $C_{15}H_{18}O_7$ (Paternò and Oglialoro), is prepared by the action of hydrochloric acid on the ethereal solution of picrotoxin, picrotoxinin or picrotoxide, $C_{15}H_{16}O_6$, being simultaneously formed:

$$C_{30}H_{34}O_{13} = C_{15}H_{18}O_7 + C_{15}H_{16}O_6.$$
Picrotoxin.   Picrotin.   Picrotoxinin.
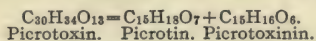
Picrotin is also formed when a chloroform solution of picrotoxin is allowed to stand in the cold, while by boiling an aqueous or benzene solution of picrotoxin that substance is split

decomposition-products of true picrotoxin, and their conclusions are confirmed by the researches of Schmidt and Löwenhardt. These latter chemists point out: (1) That true picrotoxin melts constantly at 199 to 200°; (2) that picrotoxin does not lose weight when heated to 100°, whereas picrotoxinin loses its water of crystallisation (1 molecule); (3) that picrotoxin, when treated with a large quantity of benzene in the cold, expands, whereas neither picrotin nor picrotoxinin expand in the least; and (4) that picrotoxinin, so treated with benzene, undergoes no change in its composition, which fact would scarcely be the case were it a *mixture* of the two, picrotoxinin being soluble to the extent of 3.5 in 1,000 of benzene, while the solubility of picrotin is only 0.2 in 1,000 of the same solvent.

The appearance and crystalline form of picrotoxin vary with the conditions of separation. Thus, if a fairly concentrated solution be evaporated slowly, the picrotoxin separates in well-defined prisms; but if the solution be evaporated rapidly and cooled quickly, character-istic feathery forms are deposited. When the solution is dilute, picrotoxin separates in long radiating needles. According to the United States Pharmacopœia, picrotoxin occurs as colourless, shining, prismatic crystals, or as a micro-crystalline powder.

Picrotoxin is odourless, permanent in the air, and intensely bitter. It melts at 200° to a yellow liquid, at a higher temperature evolves acid fumes having an odour like that of caramel, and ultimately chars.

Picrotoxin dissolves very sparingly (1: 200) in cold water, more readily in hot (1: 25), and is very soluble in boiling alcohol. It is also soluble in glacial acetic acid, amyl alcohol, and benzene, but is only sparingly soluble in ether or chloroform. It dissolves in ammonia and in acidified water.

Picrotoxin may be extracted from acidified aqueous liquids by agitation with chloroform, ether, or amyl alcohol, the first solvent being preferable. Benzene is stated not to extract it.

up into picrotin and picrotoxinin. Picrotin crystallises with varying quantities of water. When heated it darkens at 245°, and melts at 250 to 251°. It has a very bitter taste, but is not poisonous. Picrotin reduces ammonio-silver nitrate and Fehling's solution. Its solu-tion in benzene is unaffected by boiling or by hydrochloric acid, but it is readily decom-posed by alkalies. With strong sulphuric acid, picrotin develops a pale yellow colour, which changes to orange on heating.

Picrotoxinin or Picrotoxide, $C_{15}H_{16}O_6$, H_2O, is also formed by the action of hydrochloric acid on picrotoxin. It crystallises in rhombic plates, which become anhydrous at 100°, and melt at 200°. Pictroxinin is bitter and very poisonous. It dissolves readily in hot water, alcohol, ether, chloroform, and benzene. It is reduced by Fehling's solution and ammonio-silver nitrate, and gives colour-reactions similar to those yielded by picrotoxin.

See also Meyer and Bruger, Angelico, *Gazzetta*, 1906, 36, [ii], 645; *Gazzetta*, 1909, 39, [i], 296; *Gazzetta*, 1910, 40, [i], 391; *Atti R. Accad. Lincei*, 1910, [v], 19, i, 473.

The alcoholic solution of picrotoxin is neutral in reaction and lævo-rotatory, but the optical activity is very variously stated.

Picrotoxin is neutral to litmus, but appears to possess feeble acid properties, since it is not extracted from aqueous liquids by agitation with immiscible solvents in presence of alkalies.   It forms crystallisable compounds with certain of the alkaloids.

Picrotoxin is not a glucoside, but it reduces Fehling's solution gradually in the cold and more rapidly on heating, the value of K being about 20.   It also reduces silver from the ammonio-nitrate.

Picrotoxin suffers ready decomposition when boiled with alkali hydroxides.

When a mixture of picrotoxin with an equal weight of sodium hydroxide is moistened with a drop of water, a green colouration is produced, gradually changing to reddish-brown.

When treated with cold concentrated sulphuric acid, picrotoxin dissolves with bright yellow colour, darkening to orange-red on warming, and changing very gradually to reddish-brown.   The liquid exhibits a brownish fluorescence.

Pictrotoxin gives no colouration with nitric acid of 1.2 sp. gr., but it dissolves in acid of 1.4 sp. gr. to a liquid which leaves on evaporation a reddish-yellow residue, which becomes bright red when moistened with alkali hydroxide.

If picrotoxin be treated with strong sulphuric acid, and a minute crystal of niter added, the mixture gives a colouration varying from red to violet on addition of excess of strong alkali hydroxide.

A mixure of picrotoxin with cane-sugar becomes red on treatment with strong sulphuric acid.

Aqueous solutions of picrotoxin are unaffected by auric, platinic, or mercuric chloride, Mayer's reagent, tannin, potassium ferrocyanide and ferricyanide, and most other of the general reagents for alkaloids.

Picrotoxin is not precipitated by either neutral or basic lead acetate. R. Palm has pointed out (*J. Pharm. Chim.*, 1888, [v], **17**, 19) that if an aqueous or alcoholic solution of picrotoxin be shaken vigorously with recently-prepared and well-washed lead hydroxide (prepared by precipitating a solution of lead acetate with ammonia), the bitter principle is completely removed from solution.   If the precipitate be separated, dried gently, and treated with cold concentrated sulphuric acid, a bright yellow colouration will be produced, changing to reddish-violet on warming.

Picrotoxin has an intensely bitter taste, and is very poisonous. Fish appear to be specially susceptible to its action. When introduced into a very dilute solution of picrotoxin, the fish swim with uncertainty, lose their balance, and ultimately rise to the surface, lying on their sides and opening their mouths and gill-covers frequently. These symptoms, however, are by no means peculiar to poisoning by picrotoxin.

A. Wynter Blyth considers frogs to be more sensitive than fish to the effects of picrotoxin. The frogs "become first uneasy and restless, and then somewhat somnolent; but after a short time tetanic convulsions set in, similar to those observed in poisoning by strychnine, but with picrotoxin an extraordinary and highly characteristic swelling of the abdomen occurs."

In toxicological inquiries, picrotoxin will be extracted when an acidified extract of the material is shaken with chloroform or ether. From the residue left on evaporating the solvent, the picrotoxin may be dissolved out by hot water, and crystallises from the solution on concentration. Or the aqueous solution may be treated with neutral lead acetate, avoiding excess, and the filtered liquid shaken with recently prepared lead hydroxide. The precipitate may be decomposed with dilute sulphuric acid, and the picrotoxin extracted by ether, or reagents may be applied to the precipitate itself.

Minovici (*Ann. Pharm.*, 1901, **7**, 1) recommends anisaldehyde as a very delicate and characteristic reagent for picrotoxin, and one which indicates its presence not only when alone but also in the starchy mass of the fruit. A drop of sulphuric acid is added to the sample and, after 2 minutes when the saffron-yellow colour has become distinct, 1 drop of a 20% solution of anisaldehyde in absolute alcohol is introduced. A dark blue-violet ring is produced which soon becomes and then remains pure blue. The test can also be applied to dilute solutions in chloroform, alcohol or water, but the liquids should be warmed before testing. Picrotoxin may be detected by this test in solutions as dilute as 1 in 3,000 or 4,000. Many glucosides and alkaloids give colour reactions with the reagent, but the red-blue given by veratrin cannot be mistaken for the permanent blue given by picrotoxin, and the colour given by most of the other substances which react with anisaldehyde is in nearly every case a shade of red.

Cocculus Indicus, or *Anamirta paniculata*, the berries of which are the source of picrotoxin, is a small climbing shrub growing in India and the Malay Archipelago. In India the berries are employed as a drug,

and picrotoxin itself is official in the United States Pharmacopœia, having replaced the powdered cocculus of earlier editions.   Cocculus also forms an ingredient of an ointment having a very limited use. The berries have been used by fish-poachers for poisoning fish, and a preparation known as "Barlow's poisoned wheat" is stated to owe its active properties to the presence of cocculus.

From 2 to 3 grains of picrotoxin, or about 100 grains of cocculus, cause spasms and other symptoms suggestive of strychnine.   Strong contraction of the uterus has been observed.   Chloroform acts in antagonism to picrotoxin, and prevents the spasms caused by moderate doses.

In two recorded cases, cocculus berries have proved fatal to human beings.   In 1829, several men, of whom one died, were poisoned by drinking rum containing a preparation of cocculus.   In the other case, a boy aged 12 was persuaded to swallow a powder containing cocculus used for poisoning fish.   He suffered intense pain throughout the whole length of the alimentary canal, followed by fever and delirium, and died on the nineteenth day.   The *postmortem* symptoms were those of peritonitis.

Cocculus Indicus berries contain from 1 to 2% of picrotoxin, together with cocculin and anamirtin (?).[1]   The husks contain a non-poisonous alkaloid called menispermine,[2] but no picrotoxin.

Bitters and Resins of Hops.

Hops contain at least two crystallisable bitter substances of slightly acid character and at least three resins, of which two are soft and bitter and one is hard and tasteless.   Of these five substances, the two soft resins are of most importance commercially, since there is no

[1] **Anamirtin** is regarded by Paternò and Oglialoro as a product of the decomposition of picrotoxin, and not as a natural constituent of the cocculus berries.   It remains in the mother-liquor when picrotoxin is crystallised from water.   Anamirtin forms short needles, probably containing $C_{19}H_{24}O_{10}$.   When heated to 280° it chars without melting.   It is free from bitter taste, and is not poisonous.   It dissolves in water, but is only sparingly soluble in chloroform or benzene.   Anamirtin does not reduce Fehling's solution or ammonio-nitrate of silver.

Cocculin, $C_{19}H_{26}O_{10}$, is regarded by its discoverer, Löwenhardt, as probably identical with the anamirtin of Barth and Kretschy.   It crystallises in small white needles, sparingly soluble in hot water, alcohol, or ether, and insoluble in cold water or ether, but slightly soluble in cold alcohol.   It is not bitter, and it does not yield the colour-reactions of picrotoxin.

[2] **Menispermine**, $C_{18}H_{24}O_2N_2$, forms white, four-sided prisms, m. p. 120°.   It is insoluble in water, but dissolves in warm alcohol or ether.   Menispermine has no bitter taste, and is not poisonous.   It is a well-defined base with alkaline reaction.

Paramenispermine is separated from menispermine by treating the mixed bases with ether, in which the former is insoluble.   It forms quadrilateral prisms or radiating crystalline masses.   It melts at 250°, and at a higher temperature sublimes unchanged.   The crystals are insoluble in water, and nearly so in ether, but dissolve in absolute alcohol.

longer any doubt that the preservative properties of hops depend mainly, if not entirely, on the soft resins, which are also in all probability mainly responsible for the bitter flavour of brewers' hopped worts.

The crystallisable bitter acids are extremely insoluble in water, one of them being so insoluble that, when powdered crystals are laid on the tongue, no sensation of bitterness can be detected, and they can hardly, without undergoing some change, contribute appreciably to the bitter flavour of brewers' worts. There is reason to believe, however, that each of the crystallisable acids is closely related chemically to one of the two bitter resins, and therefore the chemistry of the acids—definite chemical entities, whose constitution is, in part at least, known—gives a hint as to the probable constitution of the more important resins or of some one constituent of each of these, supposing the resins to be mixtures which may well be the case with bodies so difficult to characterise.

Lermer (*Dingler's Polytech. J.*, 1863, **169,** 54) and H. Bungener (*Bull. Soc. Chim.*, 1886, **45,** 487) by different methods isolated from hops a crystalline bitter acid which was no doubt identical with what is to-day called the *β-hop-bitter acid* or *lupulinic acid,* but the systematic study of the bitter substances of hops dates from the work of Hayduck.

Hayduck (*Wochenschr. f. Brauerei*, 1888, 937) showed that the ethereal extract of hops, when freed from a wax previously described by Lermer (*loc. cit.*), contained at least three resins and two crystallisable substances. Two of these resins and both the crystallisable substances were soluble in petroleum ether, while the remaining resin—the hard, tasteless resin—was not. Hayduck extracted hops with ether, evaporated the ethereal extract and treated the residue with alcohol. A wax (Lermer's "myricin") remained undissolved. From the alcoholic solution, one of the resins, called by Hayduck the *α-resin*, was precipitated as a lead compound by addition of an alcoholic solution of lead acetate. By decomposition of its lead compound, the α-resin was obtained as a soft, bitter resin, soluble in petroleum ether and soluble, though sparingly soluble, in water and in dilute alcohol.

In the filtrate from the lead compound of the α-resin, the remaining two resins were found. These were separated by taking advantage of the fact that one was soluble, the other insoluble, in petroleum ether. The resin soluble in petroleum ether but not precipitated by lead acetate was a soft, bitter resin, soluble, though sparingly in water and dilute alcohol, and was called by Hayduck the *β-resin*, while

the hard, tasteless resin, insoluble in petroleum ether, was called the
γ-resin.

Hayduck made the further observation that, under certain conditions
microscopical crystals separated from the α- and β-resins. The crystals
from the α-resin differed notably in appearance from those yielded
by the β-resin, but both were intensely bitter substances of slightly
acid character. Hayduck's crude α- and β-resins must, therefore,
each be regarded as a mixture of at least one resin and one crystallis-
able substance, unless it be assumed that the crystallisable substances
are derived in some manner from the resins after the separation of the
latter by Hayduck's method. This assumption is scarcely tenable
in view of the marked tendency of each of the crystallisable
substances to become transformed into a resinous mass, indistinguish-
able from the resin from which the crystals were first separated.
Hayduck himself expressed the view that the resins proper were
simple polymers of the crystallisable acids, and that each of his so-
called α- and β-resins was a mixture of the true resin with unpoly-
merised bitter acid. H. Bungener (*loc. cit.*) had at an earlier date
expressed the view that the resin of hops—then supposed a single
substance—was an oxidation product of his crystalline acid (the
β-acid), and other authors have suggested that the resins may result
from the polymerisation of some oxidation product of the crystalline
acids, but the most recent work of Lintner and his colleagues supports
the theory of simple polymerisation.

Lintner and A. Bungener (*Zeitschr. f. d. ges. Brauwesen*, 1891, **14,**
357) prepared a quantity of the *α-acid* from Hayduck's crude α-resin
by repeated precipitation and decomposition of the lead compound.
They obtained it in the form of small, brownish, rhombic crystals,
soluble in alcohol to which it imparted a strong bitter flavour and an
acid reaction. Lintner and Schnell (*Zeitschr. f. d. ges. Brauwesen*,
1904, **27,** 666) subjected the α-acid to hydrolysis by boiling with alco-
holic sodium hydroxide and obtained, in addition to resinous matters,
valeric acid and a colourless, crystalline substance, melting at 92.5°.
This new substance showed no signs of resinification and was sparingly
soluble in water as well as being freely soluble in most organic solvents.
Analysis and determination of the molecular weight pointed to the
formula $C_{15}H_{24}O_4$, and the reactions of the substance indicated the
presence of a carboxyl group, a carbonyl group and one ethylenic
double bond. Lintner and Schnell regarded it as a hydroxyketonic

acid, and the α-hop-bitter acid as composed of this hydroxyketonic acid condensed with a valeric acid residue. They therefore ascribe to the α-acid the formula $C_{20}H_{32}O_5$ and, since it is probably a lactone, propose to call it *humulone*.

Lintner (*Chem. Zeit.*, 1908, **32**, 1068) has analysed the lead salt of the α-acid and of the crude α-resin (mostly resin with some unpolymerised α-acid), and finds the lead content in each case to approximate 36.65%, theory for $C_{20}H_{29}O_5Pb. O.PbC_{20}H_{29}O_5$ being 36.69%. On this observation, which supports the view that the α-resin is a simple polymer of the α-acid, Lintner has founded a gravimetric method (see Commercial Analysis of Hops) for the estimation indifferently of the α-acid or α-resin, that is to say for the estimation of the crude α-resin of Hayduck.

Lintner and Barth (*Zeitschr. f. d. ges. Brauwesen*, 1900, **23**, 509, 537, 554, 572, and 594) obtained the *β-acid* in long, massive, colourless prisms, melting at 92 to 93°, by employing methyl alcohol as solvent. The crystals were absolutely insoluble[1] in water. The formula of the β-acid was found to be $C_{25}H_{36}O_4$ and the name *lupulinic acid* has been suggested for it. Its acid character, however, cannot be due to carboxyl groups, since it may be extracted by ether from its alkaline solutions, and the bromine additive product—a tasteless substance—has no acid properties. The acid character as well as the bitter taste, therefore, seems to depend on the presence of unsaturated carbon linkages. Lupulinic acid yields valeric acid on oxidation by permanganate in alkaline solution. Other reactions point to the presence of one methoxyl group and two double linkages. Like the α-acid, it appears to be a derivative of an olefine-terpene, since both on fusion with potassium hydroxide and subsequent distillation over phosphorus pentoxide yield a hydrocarbon, C_5H_8.

Seyffert (*Zeitschr. f. d. ges. Brauwesen*, 1892, **15**, 62) has described a crystalline substance which he obtained from the *γ-resin*. This substance melted at 160° with decomposition and formation of a substance resembling the γ-resin in appearance, but it has not been further investigated. Hayduck supposed that the γ-resin was derived from the hop-oil, and Chapman has since shown (*Trans.* 1895, **67**, 54) that myrcene, one of the constituents of essential oil of

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[1] Though practically insoluble in water, lupulinic acid yields a bitter solution when boiled with water through which air is bubbled, evidently because it polymerises to a resin soluble in water. On exposure to air it is gradually transformed into a yellow resin and develops an odour resembling that of fatty acids.

hops, does very readily undergo oxidation and polymerisation with the production of a hard resin, but this resin cannot be identical with Hayduck's resin, which is tasteless or nearly so. Chapman's resin, on the other hand, possesses in a marked degree the aroma of the hop-oil itself and to it no doubt is chiefly due the characteristic hop flavour (as distinguished from the merely bitter flavour) of hopped worts. Having regard to the readiness with which Chapman's resin is formed from myrcene at ordinary temperatures, it is not improbable that it exists ready-formed in hops, but the demonstration of its presence would be attended with great difficulty and so far as the writer is aware has not been attempted. Quantitatively, it cannot be an important constituent of hops, which contain at most 0.2 to 0.3% of myrcene, whereas the total content of resins may exceed 15%.

A more probable explanation of the origin of the γ-resin is that put forward by Briant and Meacham, namely that it is derived in some manner from the soft resins or one of them. Some continental writers, in reviewing the present state of our knowledge of the resins and bitters of hops, pass very lightly over the work of Briant and Meacham, on the ground that it is questionable whether the "hard" and "soft" resins of these authors are chemical entities or mixtures of various resins with the essential oil of hops. This question will be discussed in relation to the *Commercial Analysis of Hops* by their method. But although the work of Briant and Meacham was directed to the discovery of means for the commercial valuation of hops and for arresting or retarding their deterioration on storage rather than to the isolation of chemical entities, they established one important fact which properly finds a place here. It had long been known that the deterioration of hops on storage was accompanied by a diminution in the total content of resins, but Briant and Meacham were the first to point out (*J. Fed. Inst. Brew.*, 1896, **2,** 414) that, for a time at least, the percentage of hard resins increased. In one case, where hops were analysed before and after storage, the total resin fell only from 14.9 to 14.75%, but the percentage of soft resins fell from 11.7 to 8.8, being nearly balanced by an increase in the hard resins from 3.2 to 5.95%. The precise inference to be drawn from this and similar experiments depends on the nature of Briant and Meacham's "soft" and "hard" resins. They regard these as substantially identical with Hayduck's soft (mixture of α and β) and hard (γ) resins, and conclude that, among the changes undergone by the constituents of hops on storage,

there is a gradual transformation of the α- and β-resins, or of one of these, into the γ-variety. This view is supported by the fact that old or badly stored hops are deficient in preservative value, and it has been known since the work of Hayduck that the antiseptic properties of hops depended on the α- and β-resins, the γ-resin having little or no antiseptic value. In discussing Briant and Meacham's method for the *Commercial Analysis of Hops*, it will be shown that their soft resin represents the sum of the α- and β-resins, plus a minute percentage of wax and possibly of some other substance not present in Hayduck's resins. Their hard resin conforms entirely to Hayduck's description of the γ-resin as a substance soluble in ether, but not in petroleum ether, and devoid of antiseptic properties. Neither Hayduck nor any subsequent worker has characterised the γ-resin more nearly than that. Briant and Meacham regard the transformation of the soft resins into hard resin as due to enzyme action, and, as recently as 1905, Briant ("The Hop and its Constituents,"[1] ed. Chapman, page 52) re-affirmed his conclusion that oxidation had no part in the process. This conclusion was based on the observation that the deterioration of hops was arrested by cold storage, but not by storage *in vacuo* at ordinary temperatures. In a footnote, however, Briant admits that vacuum storage (by which of course something less than perfect exclusion of air is meant) has proved more satisfactory than his own experiments led him to expect, and he himself (*J. Fed. Inst. Brew.*, 1896, **2,** 412) showed that hops could be preserved as well at ordinary temperatures in an atmosphere of carbon dioxide as they could be by cold storage. The possibility that the transformation of the soft resins into hard resin is partly, or even wholly, an oxidation process is, therefore, not excluded.

From the above review of the more important work on the resins and bitter acids of hops, it will be seen that these must be regarded as a group of closely related substances with a marked tendency to undergo transformation, and that there is some evidence for the view that this transformation proceeds mainly in the direction Crystalline Acid $\longrightarrow$ Soft Resin $\longrightarrow$ Hard Resin.

Payen and Chevalier (*J. Pharm. Chim.*, 1822, **8,** 209) and Doebereiner (*Deutsches Apothekerbuch*, [iii], 1847, 636) described a bitter substance which they isolated from hops. This substance was de-

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[1] A Monograph on the Hop Plant, repeatedly referred to in this Section. Pub. by the *Brewing Trade Review*, London, 1905.

scribed by them as a yellowish-white, neutral substance, soluble in
5 parts boiling water, less soluble in cold water and almost insoluble
in ether.   So far as the writer is aware, this substance has not been
further investigated.

Hops.

The hops of commerce are the ripened and artificially dried female
flowers of *Humulus lupulus*, a diœcious plant belonging to the order
Cannabaceæ.   The female flowers, which are very small and very
simple in character, consisting of a cup-shaped corolla with a round
ovary containing one seed, grow together in structures somewhat
resembling fir-cones and technically termed "strobiles."   On dis-
section, each strobile or "hop" will be found to consist of a number
of leaf-like bracts and bracteoles, termed by hop-growers "petals"
or "scales," arranged on a central axis or "strig."   The bracts are ar-
ranged on the strig in 4 rows and in reality are pairs of stipules of
leaves whose green leaves usually remain undeveloped.   Just above
each pair of these bracts, short branches grow out from the central
axis and upon each branch are produced 4 female flowers, each sur-
rounded by a bracteole.   The ripened hop thus consists of a strig,
n bracts, $2n$ bracteoles and $2n$ fruits or abortive ovaries, n being any
number from 8 to 50, 15 being perhaps the average for English hops.
The fertilised and ripened fruit (an achene containing a single seed)
is termed in the trade a "seed."   Continental hops are almost seedless
and even English Goldings, which do not develop well unless a fair
proportion of the flowers are allowed to become fertilised, contain a
large number of abortive ovaries, which are small, shrivelled, incon-
spicuous structures.

The bracts and bracteoles are of approximately the same size, but
differ in shape and texture.   The bracts are the more symmetrical and
are rather coarser in texture and more thickly veined than the bracte-
oles, which are usually slightly longer than the corresponding stipular
bract.   Toward its base, on one side, the bracteole is folded on itself
and encloses the fruit if one has developed.   The bracteoles are not
only longer but narrower at the tip than the bracts, the upper parts of
which are broader as a whole, though suddenly becoming acuminate
at the actual tip.   The bracteoles are also of a brighter yellow colour
than the bracts when the hop is ripe.

On examining the dissected parts of a hop-cone with a lens, a large

number of pollen-like grains will be observed attached to the outer surface of the bracteoles, especially near their base. The pericarp, enclosing the seed, is also thickly covered with them, and, in the better class of hops, they will be found on the stipular bracts as well. Botanically these structures are regarded as glandular trichomes or hairs, and they are spoken of by growers and brewers as "hop-meal," "hop-flour," "pollen," or "condition." The Pharmacopœias (British and United States) call the hop-meal "lupulin," and the expression "lupulin glands" is very general among chemists. In England, the glands make their appearance toward the end of July or beginning of August as small cup-shaped structures, which become more and more filled with oily, resinous contents as the season advances. The oily material is, for a time, transparent, of a clear golden yellow colour resembling amber varnish, but when the glands are dead ripe the contents become turbid and opaque and are not unlike flowers of sulphur. In commercial samples they always appear transparent, as hops are usually picked unripe and, even if allowed to ripen, the subsequent drying process once more restores to the glands their transparent appearance. The glands darken in colour and suffer other visible changes on storage or when dried carelessly, but a consideration of these changes is best deferred until, toward the end of this section (see Physical Examination of Hops), the physical characteristics of a good hop are considered.

For a fuller account of the morphology of the hop, the reader is referred to an article by Percival in a Supplement to the *Brewers' Journal*, 1902, 38, March).

Lupulin.

Lupulin is defined by the British Pharmacopœia as glands obtained from the strobiles of *Humulus lupulus*. It should contain not more than 40% of matter insoluble in ether, and yield not more than 12% of ash when incinerated.

The United States Pharmacopœia also requires lupulin to contain at least 60% of matter soluble in ether and places the limit of ash at 10%.

The British Pharmaceutical Codex (1907) describes lupulin at length and in particular states that the glands may be separated by shaking and beating hops and that they readily burst and discharge their granular oleoresinous contents on the application of slight pressure. It also adds the warning that the commercial article may be kiln sweepings, and that a dark colour and disagreeable odour are an indication

of age. It is further stated that some samples may contain as much as 25% of ash and more than 40% of matters insoluble in ether.

Apart from the fact that the leafy organs of the hop afford a filter bed for the separation from brewers' wort of the albuminoid and other substances thrown out of solution on boiling, the value of hops to the brewer depends, so far as is known, entirely on the contents of the lupulin glands. These glands contain not only the preservative resins and bitter acids but the essential oil on which the aroma of the hop, and indirectly the flavour of hopped worts, depend.

The percentage of lupulin in good hops approximates 20%, though it is rarely possible to separate so much mechanically. On extracting lupulin with ether, from 60 to 80% may dissolve. The ethereal extract is mainly made up of the resins and bitter substances already described, the insoluble portion of cellular tissue and sand. Barth ("The Hop and Its Constituents," ed. Chapman, page 81) gives the following analysis of a commercial sample which contained 36% of matters insoluble in ether.

	Per cent.
Soluble in ether:	
Ether-soluble ash,	0.2
Wax,	0.2
α-Resin and α-acid,	11.6
β-Resin and β-acid,	43.3
γ-Resin, essential oil, fatty oil, etc. (by difference),	8.7
	64 per cent.
Insoluble in ether:	
Ash, insoluble in hydrochloric acid,	15.3
Ash, soluble in hydrochloric acid,	2.7
Albuminoids ($N \times 6.25$)	4.8
Pentosans (by Tollens' method),	2.3
Crude fibre, tannin and loss (by difference),	10.9
	36 per cent.

With regard to the constituents not estimated, or estimated only by difference, it may be said that lupulin may contain a trace of morphine and perhaps of another alkaloid. Certain American wild hops certainly do contain morphine and another substance of alkaloidal character to the extent of 0.15%, but not more than infinitesimal traces of these substances have been found in cultivated hops (Williamson,

Chem. Zeit., 1886, **10**, 20, 38 and 147, and Ladenburg, *Ber.*, 1886, **19**, 783). Lecithin also is no doubt present in hops, though probably in small quantity in ripe hops, and would in part pass into the ethereal extract. It is probable that the cholin obtained from hops by Griess and Harrow (*Ber.*, 1885, **18**, 717) was not present as such in the hops, but resulted from the splitting up of lecithin.

Barth in his analysis groups together γ-resin, essential oil and fatty acids. Chapman (*Trans.*, 1895, **67**, 55), who submitted some hundredweights of hops of various origin to steam-distillation, obtained only about 0.2% of essential oil, equivalent to about 1% on the lupulin. The "seeds" of hops contain upward of 25% of a fatty oil, and kiln-sweepings, which are said to be sometimes sold as lupulin, may therefore contain fatty oil derived from ruptured seeds. The structure of the hop cones must protect the seeds of pocketed hops in ordinary circumstances, for crushed seeds are rarely found, but Briant (*J. Inst. Brew.*, 1910, **16**, 5) has shown that in exceptional cases the seeds may be ruptured. Barth's lupulin, however, was specially selected for use in an investigation on hop resins and it is improbable that it contained any considerable amount of oil derived from seeds. His figure for "γ-resin, etc.," less 1% for essential oil, may be taken, therefore, as an approximate measure of the γ-resin as defined by Hayduck.

The insoluble ash of lupulin varied from 10 to 20% in a number of samples examined by Barth (*loc. cit.*), while at least one sample has been described as containing only 4% of total ash. Tannin is present in hops to the extent of 2 to 4%, but its seat is in the leafy organs, not in the lupulin glands, so that Barth's figure of 10.9% may be taken as a fair measure of the crude fibre in his sample. The crude fibre has been reported as low as 6% by at least one author and much higher than 11% by several others.

Before proceeding to the Valuation of Hops, it should be stated that hops intended for use in brewing, their chief use, are dried on kilns (oasts) over coal fires on which, in Great Britain at any rate, it is customary to throw some sulphur. The reasons for the use of sulphur will be discussed when we come to consider the *Detection of Sulphuring*. In Great Britain, drying generally succeeds picking as fast as the capacity, often inadequate, of the oasts permits. With some Continental hops, on the other hand, a very considerable time elapses between picking and curing. The dried hops are finally pressed into bales (pockets) and are then ready to be marketed.

Valuation of Hops.

Hops are valued by their chief user, the brewer, for the preserving effect they have when added to beer, for the peculiar bitter taste they impart to the latter and; last but by no means least, for their aroma. It might be thought, therefore, that the labours of the commercial analyst would be directed to the estimation of the constituents of the hop on which these properties depend. The estimation of the soft resins, on which the preservative properties and bitter flavour depend, is in fact one of the most important aids that the chemist can give to the brewer in the selection of a hop, but as regards the essential oil the case is very different, and there is little doubt that it is the quality rather than the quantity of the essential oil which is most important to the brewer. Since the quantity is very small (0.2 to 0.5%) and, in all samples reported on, made up as to 80 to 90% of 2 hydrocarbons, the differences in different samples being apparently due to variation in proportion of the minor constituents, it will be obvious that the chemist's task would be a very difficult one, even if we knew which of the minor constituents contributed to the delicacy or rankness of odour which distinguishes hops of different origin and growth. As a fact, we have not yet this knowledge, and the nose of the buyer remains his sole guide in the matter of aroma.

Chapman (*Trans.*, 1895, **67**, 54; 1903, **83**, 505) fractionated the oil from several hundredweights of hops of different origin and found that each sample yielded 40 to 50% of an unsaturated aliphatic hydrocarbon, boiling at 166 to 168°, two small intermediate fractions, and about 40% of a sesquiterpene boiling at 263 to 266°. The sesquiterpene, to which Chapman gave the name of *humulene* (*Trans.*, 1895, **67**, 780), possesses little odour. The intermediate fractions consisted mainly of inactive linalool and linalyl isononoate, both of course fragrant substances, while the unsaturated aliphatic hydrocarbon, constituting the first fraction, was subsequently shown to have the formula $C_{10}H_{16}$ and to be identical with *myrcene*, first obtained by Power and Kleber from bay oil. Myrcene has a penetrating odour which is not unpleasant but which is quite distinct from that of the unfractionated hop oil. Essential oil of hops also contains a small amount of a diterpene, probably some ester of geraniol, and not improbably other esters.

The smell in the neighbourhood of a brewery would suggest that little of the essential oil of hops can remain in the brewer's wort at the end of the boiling period, and Chapman ("The Hop," page 66) has

shown that, even after half an hour's boiling, 80% of the oil has vola-
tilised, the great bulk of the remaining 20% becoming converted into
a sticky resinous mass. This resin results mainly from the oxidation
and polymerisation of the myrcene and it is important to note that it
possesses in a marked degree the aroma of the oil itself and that it is
appreciably soluble in brewer's wort. To this resin is no doubt
largely due the characteristic flavour of brewer's hopped wort and of
the resultant beer. Brewers add hops not only to their boiling wort
but also to some of their finished beers when in the trade casks, using
for this purpose always the finest hops, and, as a result of this "dry-
hopping," a very small quantity of essential oil does no doubt find
its way unchanged into the beer and contribute to the flavour. But
what determines the difference in flavour of say Californian, Bavarian
and Kentish hops is unknown. Even an inexperienced person can at
once recognise that their odours are very dissimilar, yet Chapman
isolated the same constituents in not very different proportions from
oil derived from each of these.

So far, therefore, as the valuation of hops depends on the report
of the chemist, his estimation of the soft resins will have most impor-
tance for the buyer. He is also called on to determine moisture in
hops, since hops containing an excessive amount of moisture do not
keep well, and he is frequently asked to decide whether hops have
been subjected to the action of sulphur dioxide on the kiln and whether
they contain free sulphur, derived from spraying mixtures used during
the growing period to keep down mould. Every sample of hops sub-
mitted to the analyst should be examined for traces of arsenic, derived
from the fuel used during the drying process.

Important as it is to the brewer to know that his hops possess ade-
quate preservative properties and that they are free from arsenic and
excessive moisture, yet enormous dealings in hops take place as the
result of mere hand examination. A brief description of the physical
characteristics of a good hop follows the analytical section.

Commercial Analysis of Hops.

Estimation of Soft and Hard Resins.—In Great Britain, this is
usually carried out by the method of Briant and Meacham, first de-
scribed by these authors in 1897 (*J. Fed. Inst. Brew.*, 1897, **3**, 233),
but in use by them and others at their suggestion long before that date.

A weighed quantity (3 or 4 grm.) of the hops is placed in a paper thimble in a 100 c.c. Soxhlet apparatus and is extracted with light petroleum (b. p. about 50°) for a period of 24 hours. The petroleum ether extract is finally filtered free from particles of hop, the bulk of the solvent distilled off, and the residue of *soft resins* dried to constant weight in an oven maintained at about 60°.

After removal of the soft resins from the hops with petroleum ether, the hops are extracted for 12 hours with ordinary ether. The ethereal extract is treated precisely like the petroleum ether extract and the dried extract is weighed and returned as *hard resin*.

Another method in use in Delbrück's laboratory before 1897 and still in use in at least one British brewing laboratory, differs little from the method by which Hayduck first differentiated the resins, except that it does not seek to distinguish between the two soft (α- and β-) resins. The hops are first extracted with ordinary ether, which removes all the resins. The ether is evaporated, the residue taken up in 90% alcohol to separate the wax, the alcoholic extract of the three resins evaporated, and the α- and β-resins separated from the γ-resin by extraction of the former with petroleum ether.

Remy (*Wochenschr. f. Brauerei*, 1898, 530) has improved on this method as follows: After separation of the wax as described, the alcoholic solution is evaporated to dryness, the residue again dissolved in ether and the ethereal solution repeatedly shaken out with 0.5 % sulphuric acid to remove certain basic substances. The ethereal solution is finally evaporated and the hard and soft resins are separated by means of petroleum ether as before described. Remy dries the resins for 6 hours at 90°.

It has been said (*e.g.*, by Barth, "The Hop," ed. Chapman, page 78) that it is questionable whether Briant and Meacham's hard and soft resins are chemical entities or mixtures of various resins with essential oil. There can be no doubt that the soft resin of Briant and Meacham contains essential oil, but so no doubt did the β-resin of Hayduck. Good hops contain more than 10% of soft resin, less than 0.5% of essential oil, and the unavoidable error of sampling hops for the determination of resin makes the result of this determination uncertain by as much as 0.5%. Hops contain very much less than 0.1% of wax and there is no evidence that Remy's basic substances are present in quantity. Certainly Hayduck knew nothing of them, and, in the great majority of cases, the soft resins obtained by the method of

Briant and Meacham do, for all practical purposes, represent the α- and β-resins of Hayduck, and their hard resin his γ-resin.

Occasionally, but very rarely, a sample of hops may be received which contains a large number of crushed seeds. Briant and Harman (*J. Inst. Brew.*, 1910, **16,** 5) record the receipt of such a sample. Since the seeds contain upward of 25% of a fatty oil, there is risk in such a case of the resins being overestimated by either of the methods given, for Briant and Harman (*loc. cit.*) describe the oil as a drying oil and, if it resembles other known drying oils at all closely, treatment with alcohol would not leave it all with the wax.

One reason which inclines the reviser of this section to avoid methods which first extract all the resins with ether and then differentiate them by means of petroleum ether is the fact that the dried ethereal extract will seldom redissolve completely in ether, indicating that some change takes place during evaporation and drying, even when these operations are conducted at low temperatures.

A volumetric method for the estimation of the soft (α- and β-) resins has been described by Lintner (*Zeitschr. f. d. ges. Brauwesen*, 1898, **21,** 407). The hops (10 grm.) are boiled for 8 hours under a reflux condenser with about 300 c.c. of petroleum ether (b. p. 30 to 50°) contained in a flask marked to contain 505 c.c. The flask should be immersed to a depth of 2 to 3 cm. in a water-bath kept at 50°. When cool, the contents of the flask are made up with petroleum ether to the 505 c.c. mark, assuming, as is tolerably accurate, that the extracted hops occupy about 5 c.c. The liquid in the flask is filtered and 100 c.c. of the filtrate, corresponding to 2 grm. of hops, is mixed with 80 c.c. of 96% alcohol and titrated with $N/10$ alcoholic potassium hydroxide, 10 drops of a 1% solution of phenolphthaleïn being used as indicator. A blank experiment is of course necessary to determine the alkali required by the petroleum ether and alcohol. The α- and β-resins have the same neutralisation equivalents as the corresponding crystallisable acids and, as the β-resin usually predominates, Lintner calculates the total soft resins in terms of the β-acid ($C_{25}H_{36}O_4$; eq. 400), each c.c. of $N/10$ alkali corresponding to 0.04 grm. The results are said to agree well with those obtained by the method of Briant and Meacham.

More recently Lintner (*Chem. Zeit.*, 1908, **32,** 1068) has described the following method for the separate estimation of the α- and β-resins, which method may prove important since Barth has shown

(*Zeitschr. f. d. ges. Brauwesen*, 1901, **24,** 333) that the β-resin is a more powerful antiseptic than the α-resin. The hops (10 grm.) are extracted for 8 to 10 hours in a Soxhlet apparatus with petroleum ether (b. p. 30 to 50°). The petroleum ether is evaporated off below 40° by the use of the pump, the pressure being finally reduced as far as the pump allows. The residue (α- and β-resins) is weighed and then dissolved in methyl alcohol and the solution made up to 100 c.c. In 10 c.c. of this solution, the α-resin is precipitated by means of a 1% solution of lead acetate in methyl alcohol, care being taken to avoid the use of too great an excess of the reagent; addition is stopped when, on placing a drop of the resin solution on a doubled piece of filter-paper, the lower sheet of the paper is coloured brown by sodium sulphide solution. The precipitate is collected in a Gooch crucible, washed with methyl alcohol and ether, dried for an hour at 80 to 90°, and weighed. The precipitate consists of the lead compound of the α-resin and contains on the average 36.65% of lead, almost exactly equal to the calculated lead content (36.69%) of the lead compound of the associated crystallisable acid, $C_{20}H_{29}O_5Pb.O.PbC_{20}H_{29}O_5$. The figure 36.7 is used in calculating the α-resin.

Good, new hops, according to their origin and the season, may contain from 11 to 16% or even more of α- and β-resins, and 3 to 6% (usually 3 to 4%) of γ-resin, as determined by the method of Briant and Meacham. In three years under average storage conditions where no cold store is available, the sum of the α- and β-resins may fall as low as 4%, and the percentage of γ-resin may rise to more than 10, but the increase of γ-resin never quite equals the decrease of the others, so that the sum of the resins tends to decrease and in some cases decreases rapidly. In Great Britain to-day, however, hops are usually cold-stored and the deterioration is then very slow. Hops have been kept in cold store for 5 or 6 years without the loss of even 0.5% of soft resin. Regarded as an antiseptic, such hops still possess a high value, but it must not be supposed that their commercial value is equal to that of good new hops, for during so long a period of cold storage the aroma is to a considerable extent lost.

Very few figures are available as to the relative proportions in which the α- and β-resins may occur, but, so far as they go, they point to the β-resin as predominating. Its amount seems rarely to be less than twice that of the α-resin and may be four times as great.

In judging hops on their resin content, it should not be forgotten

that, though soluble, the soft resins are but sparingly soluble in brewers' wort and in beer, and that brewers' spent hops not infrequently possess marked antiseptic properties. It may well be, therefore, that there is some reasonable percentage of soft resins, the possession of which by any hop makes it, as a practical antiseptic, the equal of even more resinous samples.

Direct Estimation of the Antiseptic Power of Hops.—Quite recently, Brown and Ward (*J. Inst. Brew.*, 1910, **16**, 64) have described a biological method for the direct estimation of the antiseptic power of hops, a method which should prove useful when further experience has been gained with it. Brown and Ward make use of an organism which they isolated from a sample of acid beer and which, for the moment, they designate *Bacterium "X."* *Bacterium X* possesses many of the morphological characteristics of Van Laar's *Saccharobacillus pastorianus*, but is apparently not identical with that species. As an aid in the practical valuation of hops it possesses two useful characteristics, namely a capacity for rapid multiplication in suitable liquid media and a pronounced intolerance of hops or their aqueous extracts. Professor Brown (Birmingham University) is, for the present, kindly supplying cultures of this organism to any interested persons.

The most suitable nutritive medium for the test is perfectly brilliant cold-water extract of malt, which is made as follows. Equal measures of ground pale malt and of distilled water are digested at room temperature for two hours and the mixture filtered. It is important that the extract be made and kept brilliant. To this end it is boiled for at least an hour in a wide beaker (not a flask), re-filtered, cooled and diluted to a sp. gr. of 1,030. If not perfectly brilliant, it is fined with algin and isinglass finings and re-filtered. Test-tubes are then charged with 10 c.c. of the brilliant extract, plugged with cotton wool and sterilised for not more than 10 minutes.

A 1% aqueous extract of each of the samples of hops it is wished to compare is made by heating 5 grm. of each sample with 500 c.c. of distilled water for one hour in a steam steriliser and subsequently filtering the extracts.

Varying amounts of each extract are measured by means of a graduated pipette into a series of the test-tubes containing the cold-water malt extract. The replugged tubes are then sterilised for not more than 10 minutes. When cool, each tube is inoculated with a drop of a freshly grown, vigorous culture of *Bacterium X*, and the tubes are in-

cubated for 18 hours at 30°. If the quantities of hop extract taken have been well chosen, each set will contain one or more tubes in which a marked growth will be visible, while there will be at least one tube in which the eye can detect no turbidity. An example may be given from Brown and Ward's paper.

Oregons, 1908		Mid-Kents, 1908	
Volume extract used, c.c.	Observation	Volume extract used, c.c.	Observation
0.5	Strong growth.	1.0	Strong growth.
1.0	Trace of growth.	1.5	Strong growth.
1.5		2.0	Small growth.
2.0	No growth.	2.5	Trace of growth.
2.5		3.0	No growth.

The limit of toleration possessed by the organism would appear to be about 1.25 c.c. of the Oregon extract or 2.75 c.c. of the Mid-Kent. Since even 0.4 c.c. of a 1% extract of some hops has been found to arrest the development of *Bacterium X* under the experimental conditions described, it is proposed to refer the "antiseptic power" of hops, as determined by this method, to an arbitrary scale according to which a hop will be said to have an antiseptic power of 100 when 0.25 c.c. of a 1% extract of it, added to 10 c.c. of cold-water malt extract, is just sufficient to arrest the development of *Bacterium X*. On this scale the Oregon hop referred to would be credited with an antiseptic power of $\left(\dfrac{100 \times 0.25}{1.25} = \right)$ 20, the Kent with an antiseptic power of 9. These hops were two years old and not of high quality. Oregon and Bavarian hops of good quality may give values ranging from 60 to 80 or more. The method has not yet been applied to many good English samples or indeed to many samples of any kind, but the best values given by Brown and Ward for English hops lie between 30 and 40.

The trustworthiness of the method has been confirmed by comparing the toxic action of a 3% extract of hops showing an antiseptic power of 30, by the standard method, with a 9% extract of hops showing an antiseptic power of only 10. When these extracts were substituted for the standard 5% extract in the test, substantially identical results were obtained.

It should be added that no method analogous to the Rideal-Walker

method for testing disinfectants is applicable to hops, since the hop resins are not disinfectants, that is to say they do not kill, but merely inhibit the growth of bacteria—at any rate of such bacteria as cause trouble in beer. In one direction Brown and Ward's method will, before it receives general adoption, need to be approximated to that of Rideal and Walker. The virulence of the cultures of *Bacterium X* used by different observers, or even by the same observer on different days, will presumably vary and may vary widely. It will be necessary, therefore, to make the experiment always a comparative one, using perhaps a dilute solution of salicylic acid or of potassium metasulphite in place of the phenol of the Rideal-Walker test.

Brown and Ward give no account of the resinous constituents of the hops used in their experiments. Fine Oregons and fine Bavarians usually contain a high percentage of soft resin, but not anything like twice as much as is found in English hops of average quality. Brown and Ward's English samples may have been below average, but it is at least as likely that in Oregon hops the ratio of β- to α-resin is higher than in English hops. It has been stated that this ratio may vary from 2:1 to 4:1 and possibly even more widely, very few data being available, and the β-resin is known to be a more powerful antiseptic than the α-resin.

Brown and Ward's method is open to the same criticism as attaches to estimates of brewing value based on the percentage of soft resin, namely, that the brewer not uncommonly uses more hops than he needs for preservative purposes and that in consequence an antiseptic power in excess of some sufficient amount has no value for him. That criticism does not touch the principles of their method, however, merely limiting the judgment to be based on its results. To make practical use of Brown and Ward's method, what we need to know is what antiseptic power on their scale, or preferably on a scale which does not depend on the state of health of a living organism, constitutes a sufficiency for the brewer. Beyond that sufficiency, no marks should be awarded.

Estimation of Moisture.—This is carried out in many laboratories by drying 3 grm. of the hops *in vacuo* over sulphuric acid. If the necessary apparatus is available, the method is a good one, but almost identical results may be obtained by drying in a steam oven, a minimum weight being reached as a rule in 3 hours or less. Those who prefer the use of a vacuum desiccator usually base their preference on the loss of essential oil with the moisture at the temperature of the steam oven.

But some, at least, of the essential oil is given up even in the cold *in vacuo*, and the percentage of essential oil is very small in relation to that of moisture and certainly less than the unavoidable error of sampling.

The drying process, as usually conducted in England, reduces the moisture in hops from 60 or 70% to less than 6%. By subsequent exposure on the packing floor, the moisture always rises somewhat and, if the time of exposure be long and the atmosphere very moist, it may rise to 12% or more. Such hops do not keep well, and hops containing more than 11% of moisture should not be bought except for immediate use and, of course, only if suitable in other respects. On the other hand, hops containing much less than 8% of moisture are inclined to be brittle and scarcely bear handling without risk of falling to powder. Not only does such breaking up imply risk of loss of lupulin, but the brewer has a preference for whole hops for other good and sufficient reasons.

Detection of "Sulphuring."—Some confusion exists on this subject in the text-books, which use this term indifferently to designate two entirely different practices. In Great Britain a certain quantity of sulphur is usually thrown on the fire of the drying kilns toward the end of the drying process. The resulting sulphur dioxide tends to restrict the subsequent development of moulds and other objectionable micro-organisms, though the concentration of the gas in the atmosphere of the kiln can hardly destroy all the spores. So far as it acts as an antiseptic, its action is of course useful, but the grower's chief motive for the use of sulphur on the kiln is the improvement it effects in the appearance of his hops. If this misleads the brewer in his choice of hops, the practice is not free from objection, but English brewers are good judges of hops, and possibly the antiseptic value of sulphur dioxide outweighs the objection attaching to the production of a fictitious appearance. Brewers here and there attribute occasional yeast difficulties to the use of heavily sulphured hops, but the evidence of causal connection is seldom very strong when investigated. However, sufficient prejudice exists in certain quarters for requests to be made to the analyst to determine whether or no a particular sample of hops has been sulphured and some continental works devote considerable space to the subject.

Prior (*Chemie u. Physiologie d. Bieres*, Leipzig, 1896) gives the following directions for the detection of sulphuring. The hops (10 grm.)

are extracted in the cold with 200 c.c. of distilled water in a large flask and the mixture is frequently shaken during half an hour, at the end of which time it is filtered. Of the clear filtrate, 50 c.c. is brought into a 150 c.c. Erlenmeyer flask together with 1.5 grm. zinc and 25 c.c. hydrochloric acid (sp. gr. 1.125). The mouth of the flask is loosely plugged with bibulous paper, of which the portion facing down the neck of the flask is moistened with a solution of basic lead acetate. At the end of half an hour the plug is removed and examined. According to Prior, the presence of only 1% of freshly sulphured hops in the sample is sufficient to give rise to a distinct brownish-yellow colouration of the plug under the conditions of the test. This is said to correspond to at least 0.07 mgrm. SO_2 in the 50 c.c. of hop extract (= 2.5 grm. hops) operated on, or 0.003% on the sample. The prescribed conditions must be strictly adhered to since, if the evolution of hydrogen be too violent, most hops will yield hydrogen sulphide (Lintner, *Chem. Zeit.*, 1908, **32**, 1068).

If several reduction experiments be made with the same hop extract and the several plugs examined after 3, 5, 8, 10, etc., minutes respectively, an approximate measure of the extent of sulphuring may be obtained. A distinct discolouration within 5 minutes is said by Prior to correspond to 0.012% of SO_2 in the sample, within 3 minutes to 0.15%. Heavily sulphured hops, fresh from the kiln, may contain as much as 0.3% SO_2, but under ordinary storage conditions at least half of this disappears within a month, and in the course of very few months falls to 0.07%, at which it remains fairly stationary for at least a year.

Detection of Free Sulphur.—The other practice referred to as "sulphuring" is that of dressing the growing hops with flowers of sulphur to arrest the development of mould. This is often the only available means of saving the crop and no argument like that sometimes directed against the use of sulphur in the kiln, that it is a practice the benefit of which is wholly on the side of the grower, can be brought against it, for lost crops mean high prices which hit the brewer. But it has been said that, when hops have been heavily sulphured in the field, their use in the brewery has given rise to "stench," owing to the sulphur finding its way to the fermenting vessels and, in contact with living yeast, being reduced to hydrogen sulphide. It is possible for sulphur, as such, to find its way on to the hops during the kilning process also, if the sulphur used in that process be unskilfully applied. For the detection of sulphur in hops, according to the so-called Burton

method, 5 grm. of the hops are heated with 250 c.c. of water and 5 grm. of slaked lime in a beaker which is placed in a boiling water bath for 20 minutes. A portion of the solution is filtered, cooled and tested at once with a freshly prepared dilute solution of sodium nitroprusside. Nearly all English hops, when tested in this manner, give a red colouration. An experienced worker may decide from the intensity of the colour whether the hops can properly be described as containing notable or excessive amounts of free sulphur, but the chemist turning to this book for information will presumably not have experience of hops and their sulphur content. The colouration is transient and could not easily be made the basis of a quantitative method with any pretensions to accuracy, but solutions for comparison could be made up from sulphur-free hops and weighed amounts of sulphur. The necessary sulphur-free hop is more likely to be found among a collection of Oregons or Bavarians than among the English samples which may be available.

For the quantitative *determination of free sulphur* in hops, the following method is preferable: The hops are extracted with xylol in a Soxhlet or other extractor, a piece of bright copper foil (reduced in hydrogen) having been first placed in the flask and weighed with it before adding the xylol. The xylol dissolves the sulphur, which at the temperature of boiling xylene combines with the copper to form copper sulphide, which in small quantity is fairly adherent. A dozen syphonings are more than sufficient. The foil is washed a few times by decantation with small quantities of xylol, then with petroleum ether, and the flask dried in a steam oven, cooled and weighed. The increase in weight gives the amount of sulphur in the hops taken for the experiment. Large quantities of copper sulphide tend to flake off and might be lost in washing, but hops seldom or never contain enough sulphur to give rise to flaking.

Estimation of Arsenic.—English hops almost invariably contain detectable traces of arsenic derived from the fuel used for drying them. The Royal Commission on Arsenical Poisoning, in their final report issued in 1903, recommended that the Board of Inland Revenue should prescribe for the different materials used in the preparation of beer an adequate test by which their freedom from arsenic might be ascertained. The Commission further expressed the opinion that no substance should be used as a food or as an ingredient of food which contained more than 1/100 grain of arsenic per pound or per gallon,

according as it was a solid or a liquid. Perhaps 50 out of every 100 samples of English hops contain arsenic in excess of this amount and it is argued by hop merchants that the recommendation of the Commission is unreasonable as applied to hops which are used in relatively small quantity in beer, namely, 1 to 5 pounds per barrel. If the hops were the sole source of arsenical contamination, therefore, even the use of 5 pounds of hops containing 1/20 grain of arsenic per pound would only introduce 1/4 grain of arsenic into the barrel or much less than the allowable 1/100 grain per gallon. But hops are not the sole source of arsenical contamination. Malt, the principal raw material of the brewer, always contains detectable traces of arsenic, and it is not rea-sonable to require the maltster to deliver it with less than 1/500 grain per pound, though he frequently succeeds in doing so. Brewing sugars, too, are not quite free from arsenic, though in those of British manu-facture the amount is, as a rule, extremely minute. Since in strong ales as much as 3.5 pounds of malt may be used per gallon, the use of malt containing 1/500 grain of arsenic per pound might introduce as much as 0.007 grain of arsenic into each gallon. If there is to be certainty that the finished beer shall not contain more than 0.01 grain per gallon, not more than 0.003 grain must be allowed to find entrance with the other materials. Such a strong ale will probably be heavily hopped at the rate say of 5 pounds per barrel or 0.14 lb. per gallon, and it will be clear that the hop used should not contain more than 1/50 grain of arsenic per pound. It is fortunately true that a variable, but usually notable, proportion of the arsenic introduced with the materials is elimi-nated by the yeast, but the brewer needs to be on the safe side. He has compelled the maltster to guarantee less than 1/300 grain of arsenic in his material, and it seems unreasonable to try to stiffen this guarantee in order to free hop growers from the obligation of conducting their kilning operations with reasonable care. For the extent of arsenical contamination can be kept down by careful selection of fuel and by proper application of sulphur to the kilns if sulphur must be used. When coal is burned, some of the arsenic is volatilised, but some— with many fuels, the larger proportion—remains with the ashes. It has been shown by Baker and Dick (*J. Soc. Chem. Ind.*, 1904, **23,** 174) that, when sulphur is thrown on the fire, the arsenates in the ash may be reduced with the result that from two to four times as much arsenic may be volatilised and reach the goods on the kiln as is the case when sulphur is not used. Since special stoves for burning the sulphur apart

from the coal fire are available (*e.g.*, Eng. Pat. 16,992 of 1904, which also avoids the volatilisation of unoxidised sulphur), there seems no excuse for hop growers marketing hops with more than ten times as much arsenic as is commonly found in malt. It may be admitted that the Commission's recommendation of 1/100 grain, as applied to hops, represents a counsel of perfection rather than a standard to be rigidly enforced, but the writer has no sympathy for the grower who wants to be allowed to deliver hops containing 1/25 grain of arsenic per pound, and he would resolutely refuse to accept hops containing notably more than 1/50 grain, provided the requirements of the brewery could be filled elsewhere.

The estimation of arsenic in hops is carried out by means of the modified Marsh-Berzelius method (Vol. 1, page 147) or by the electrolytic method of Thorpe (*Trans.*, 1903, **83**, 974). If organic matter is not previously destroyed, the arsenic may be underestimated, but it is the practice in many brewing laboratories to omit this tedious procedure (Vol. 1, page 148). The brewer's object is to exclude highly arsenical consignments and, provided he is right in assuming that at least two-thirds of the arsenic is recovered when omitting the previous destruction of organic matter, he avoids risk of serious contamination of his beer and any complaint addressed to the hop merchant has an added weight if it be communicated to him that the analytical method on which the complaint is based is one that cannot overestimate and may considerably underestimate the amount of arsenic actually present.

For the destruction of organic matter, Thorpe (*loc. cit.*) recommends moistening 5 grm. of the hops with lime-water, adding 0.5 grm. of lime or magnesia, mixing thoroughly, and then heating over a low Bunsen flame until the organic matter is completely charred. The dish containing the charred residue is further heated, preferably in a muffle, until practically all the carbon is burned off. When cold, the ash is moistened with water, 20 c.c. of sulphuric acid (1:7) is added, the dish warmed and its contents transferred to a flask. About 0.5 grm. of potassium metasulphite is added and the solution boiled until free from sulphurous acid. When cool, the whole or an aliquot portion of the liquid is transferred to the electrolytic Marsh apparatus. The deposit obtained is compared with a series of standards prepared in a similar way from arsenic-free (air-dried) hops and measured volumes of a very dilute solution of arsenic.

From their bulky nature, it is impossible to moisten hops except by

the use of a large quantity of lime-water, and very difficult to secure adequate mixture with the lime or magnesia. The acid method for the destruction of organic matter as recommended by Gautier (*Bull. Soc. Chim.*, 1902, **27**, 1030) is, therefore, preferable.

10 c.c. of nitric acid and 5 c.c. of concentrated sulphuric acid are mixed in a 3.5 in. crucible and the hops added in small portions at a time, each quantity being thoroughly disintegrated by pressure under the acid with a glass rod, a further quantity of 5 c.c. of nitric acid being added when about half the hops have been thus introduced. The crucible, with its contents, is then cautiously warmed so as to avoid frothing over. When the evolution of dense red fumes ceases, the heating is increased and continued until all the acid is expelled, leaving in the crucible a black, nearly dry, charred mass. The crucible is about half filled with water and a few c.c. of hydrochloric acid (or of dilute sulphuric acid, if the electrolytic apparatus is to be used), the whole being allowed to extract for half an hour on the water-bath. The acid solution is filtered, the charred mass washed with hot water and the filtrate concentrated to about 30 c.c., which is cooled and used for the test. It is essential that the mass should be thoroughly charred and that the solution, when filtered, should be colourless. If less than 5 grm. of hops are taken, the quantities of acid recommended above may be reduced, but the use of 5 grm. enables a more representative sample to be obtained and, if the amount of arsenic in the hops is so large as to give rise to mirrors not easily compared with the standards, the final solution may be made up to 50 c.c. and an aliquot portion taken for the test.

Estimation of Tannin in Hops.—Formerly some few chemists and brewers attached great importance to this estimation, but Chapman (*J. Inst. Brew.*, 1909, **15**, 360) has demonstrated beyond doubt that the tannin of hops plays a much less important part in the brewing process than was at one time supposed and that the estimation of its amount, in the present state of our knowledge, gives no guide as to the brewing value of a particular sample. For this reason the consideration of the estimation of this natural constituent of hops has been postponed until after due account has been taken of such commonly occurring impurities as free sulphur and arsenic. The estimation is still asked for by some brewers, however, on the ground that a high percentage of tannin indicates a good hop and a hop that is not old, and that the tannin serves a useful purpose as a precipitant of albumin-

oid matters in the brewer's copper. That hops introduce almost as much soluble nitrogenous matter into the wort as they remove from it had been pointed out by earlier workers (*e.g.*, Briant and Meacham, *J. Fed. Inst. Brew.*, 1897, **3,** 482) and has been demonstrated more exactly by Chapman (*loc. cit.*). Chapman has also shown that there is no causal connection between age and percentage of tannin, and there is no evidence of any such connection between percentage of tannin and the brewing value of hops. It should be added that all early work on hop tannin is vitiated by the method used to ascertain its amount, usually a modification of the Loewenthal method (Heron, *J. Fed. Inst. Brew.*, 1896, **2,** 162), the best method in its day, but one which, in addition to other disadvantages, assumes that the whole of the substances removed by the gelatin is tannin. If the estimation is to be made, it should be made by Chapman's method (*J. Inst. Brew.*, 1907, **13,** 646) which does rest on scientific principles. (See Vol. 5, p. 91.)

10 grm. of the hops are put into a flask marked at 508 c.c. (8 c.c.= estimated volume of 10 grm. of hops); 400 c.c. of boiling distilled water is added and the hops are macerated with the aid of a stout glass rod, suitably bent at the end. The flask with its contents is then immersed for 2 hours in a water-bath, which is kept gently boiling. The contents of the flask are cooled, made up to 508 c.c. with cold distilled water, and filtered. 50 c.c. of the clear filtrate is evaporated slowly in a small beaker, which is allowed to stand on the top of a boiling water-bath. If the extraction and concentration be conducted as described, no trouble will be experienced from conversion of the hop tannin into a reddish, insoluble substance, to which the name "phlobaphene" has been given, but in regard to which little is known beyond the fact that it is readily formed when solutions of hop tannin are boiled and that regard must be had to this fact in any method for the estimation of hop tannin. When the 50 c.c. of clear extract has been concentrated to about 15 c.c., it is cooled and the tannin precipitated by addition of 50 c.c. of a saturated solution of cinchonine sulphate (about 1.5%). After standing for 1 or 2 hours the precipitate is filtered off on asbestos in a Gooch crucible. In preparing the Gooch, the asbestos should be washed with a 0.5% solution of cinchonine sulphate, pumped as dry as possible and dried to constant weight in a steam oven. The precipitate of cinchonine tannate is poured into the Gooch and allowed to filter, at first without the aid of a pump. When about half the

liquid has run through, suction is applied and the whole of the precipi- tate transferred to the crucible. When all the liquid has filtered through, the precipitate, which is much less soluble in cinchonine sul- phate solution than in water, is washed several times with a 0.5% solution of cinchonine sulphate. Care should be taken not to allow the precipitate to dry on the sides of the beaker, as it then becomes difficult to remove. When washing is completed, suction is continued until the cake of precipitate is moderately dry, as shown by its tendency to separate into several portions. The crucible with its contents is then dried to constant weight in a steam oven.

Chapman has shown that the compound of cinchonine with pure gallotannic acid contains 63% of its weight of gallotannic acid, corre- sponding to 2 molecules of gallotannic acid to one of cinchonine. The nitrogen content of the precipitate obtained from hop extracts corre- sponded to 42% of cinchonine, the balance of the precipitate (58%) being, therefore, hop-tannin. Chapman, accordingly, proposes to multiply the weight of cinchonine tannate found by 0.6 and to return the result as hop-tannin. In carrying out the estimation in the manner described, 50 c.c. of extract, corresponding to 1 grm. hops, is taken, and the weight of cinchonine tannate found multiplied by 60 gives the percentage of tannin in the hops.

Physical Examination of Hops.

In Great Britain hops are bought for the most part by brewers as a result of hand examination, or, as it is said, are judged by the "nose" and "rub." To form a fair judgment of hops as a result of such an examination requires considerable experience, which nothing written in a book can replace. But the main points to which a brewer directs his attention may be briefly stated.

To begin with, the brewer likes a whole hop and a sticky hop. When hops are broken up, there is danger that the glands may be ruptured and their resinous contents set free, when deterioration, both in loss of aroma and conversion of soft into hard resin, is accelerated.

Good, fresh samples of individual hops, rich in lupulin, stick together and only slowly open out again when pressed tightly in the hand, although as a whole a properly dried sample is very elastic and springy.

An examination of the glands with a lens is useful. In fresh,

well-managed hops, these are of a golden yellow colour, with slightly wrinkled surfaces and oily contents which readily ooze out under pressure. With age they become more wrinkled and darker in colour, assuming finally a deep red-orange tint. Too high drying induces changes in appearance similar to those which naturally result with age.

Some brewers show a decided preference for a hop with very few seeds and, other things being equal, this is reasonable, for the seeds weigh heavy and are useless to the brewer. It has been shown by Salmon and Amos (*J. Inst. Brew.*, 1908, **14,** 309) that certain of the finest English varieties of hops, notably Goldings, do not grow out well unless a fair amount of fertilisation is allowed to take place, so that any general aversion to seeded hops, such as is shown by German brewers, would probably lead to the disappearance of these varieties as unprofitable to the grower. Their brewing value, however, is such that they will doubtless continue to be bought up readily in spite of the presence of many seeds.

There is a growing disposition to admit that, in the past, the brewer has given too much attention to the delicacy of colour of hop samples. A good sample should be bright in colour, a dull, rusty appearance being generally an indication of a damaged hop, but a brown-coloured hop is not necessarily objectionable, nor will it give increased colour in the copper if the brownness be merely due to ripeness. An exceptionally pale colour may not improbably correspond to picking while still unripe, which means before full development of the preservative resins, but some brewers show a decided preference for pale hops. The colour of the foliage leaves which may be found in the sample affords a guide as to the manner in which drying has been conducted. These leaves should be of a fresh, lively green colour, not dirty brownish or olive-green.

The aroma of hops is best judged after rubbing some of the sample in the hand. Here, more than in any other part of the hand examination, is experience essential and written instruction almost useless. The brewer is concerned, not merely in satisfying himself that the hops possess sufficient aroma of the right kind, but that they are free from objectionable odours, or odours which in his experience connote deterioration due to age or damp packing, or a flavour which will overpower the delicacy of other hops with which they may be used. Damp packing gives rise to a musty odour, while old hops acquire an odour not unlike that of cheese.

The practical brewer derives a good deal of information from an examination of the "side" of the cube cut out by the sampling knife, but enough has been written on a subject which is essentially one calling for practical demonstration by an expert provided with a varied collection of hops.

Detection of Hop-substitutes in Beer.

Whatever may have been the case formerly, this subject has very little importance at the present time, in Great Britain at all events, and that for three reasons.

In the first place the proportion of hop-substitutes used in Great Britain is infinitesimal. Brewers are required to record in the Excise books all the materials they use, and the returns for the year ending Sept. 30, 1909, show that 27,422[1] *tons* of hops and only 2,954[1] *pounds* of hop-substitutes were used. Against the obvious inference to be drawn from these figures, it may be said that some of the possible substitutes possess so intensely bitter a taste that they would displace more than an equal weight of hops. On the other hand, it is a fact that a large proportion of the so-called hop-substitutes are manufactured from hops, more than one patented process being worked, having for its object the separation of the preservative and flavouring constituents of the hop and the use of these by the brewer at different stages of his process.

In the second place, even if hop-substitutes found more extensive use, it is not the business of any official in Great Britain to detect the use of such substitutes, there being no law as to what the brewer may or may not put into his beer, beyond the provision of the Food and Drugs Acts that no substance which is injurious to health may be added to any food intended to be offered for sale.

In the third place, the brewer's own permanent interests forbid him to make use of hop-substitutes, and the statistics given show that, for the most part, he recognises this. The bitter of hops differs from all other vegetable bitters in its fugitive character. When the bittered liquid has left the palate, the bitter taste passes also and does not continue to affect the palate as do other bitters, some of them for a long time after the liquid in which they were dissolved has been swallowed. Beer drinkers as a class may not know this distinction between the bitter of hops and other bitters, but there is abundant evidence that

[1] In 1902 the figures were 32,373 *tons* of hops and 19, 422 *pounds* of substitutes.

they have very sensitive palates, and it may be asserted with confidence that they would quickly abandon the use of beer bittered with any notable quantity of hop-substitute, guided by their palates alone, the impression on which they would not trouble to analyse.

Allen devoted a great deal of his time and exceptional skill to the detection of hop-substitutes as may be seen by reference to a file of the *Analyst*, but with the diminishing importance of the subject it will suffice to give the following table from among the eight pages devoted to the subject in the last edition.

OUTLINE PROCESS FOR THE DETECTION OF BITTER PRINCIPLES
IN BEER.

One litre of beer is evaporated to half its bulk and precipitated boiling with neutral lead acetate, the liquid boiled for fifteen minutes and filtered hot. If any precipitate occur on cooling, the liquid is again filtered.

Precipitate contains *hop*-bitter, *caramel*-bitter, *ophelic acid* (from chiretta), phosphates, albuminous matters, etc., etc.	**Filtrate.** The excess of lead is removed by passing H_2S, and the filtered liquid concentrated to about 150 c.c. and tasted. If any bitter taste is perceived, the liquid is then slightly acidified with dilute sulphuric acid, and shaken repeatedly with chloroform.			
	Chloroform layer on evaporation leaves a bitter extract in the case of *gentian, calumba, quassia,* and *old hops* (only slightly or doubtfully bitter in the case of chiretta). The residue is dissolved in a little alcohol, hot water added, and the hot solution treated with ammoniacal basic lead acetate and filtered.	**Aqueous liquid** is shaken with ether.		
		Ethereal layer leaves a bitter residue in the case of *chiretta, gentian,* or *calumba.* It is dissolved in a little alcohol, hot water added, and the hot solution treated with ammoniacal basic lead acetate and filtered.	**Aqueous liquid,** if still bitter, is rendered alkaline and shaken with ether-chloroform. A bitter extract may be due to *berberine* (calumba) or *strychnine.*	
Precipitate contains *old hops, gentian,* and traces of *caramel* products. It is suspended in water, decomposed by H_2S, and the solution agitated with chloroform.	**Filtrate** is boiled to remove ammonia, and treated with a slight excess of sulphuric acid filtered and tasted. If bitter, it is agitated with chloroform, and the residue examined for *calumba* and *quassia.*	**Precipitate** is treated with water and decomposed by H_2S. The filtered liquid is *bitter* in presence of *gentian.*	**Filtrate** is treated with a slight excess of dilute sulphuric acid, filtered and tasted. A bitter taste indicates *calumba* or *chiretta,* which may be reextracted with ether and further examined.	The aqueous liquid, separated from the ether-chloroform, may contain *caramel-bitter* or *choline.*
Chloroform layer is examined by special tests for *gentian* and *old hop*-bitter.	**Aqueous liquid** contains traces of *caramel-bitter.*			

The table calls for one or two comments. The *hop-bitter* precipitated by lead acetate is presumably the α-hop bitter, which is known

to be more strongly bitter than the β-variety, though the latter is usually present in greater quantity. The bitter of *old hops* of the table possibly includes the β-hop bitter as well at the γ-resin, the amount of which is known to increase with age. Modern preparations of *caramel* do not as a rule yield any precipitate with lead acetate, and finally a note of warning should be issued with regard to the mere detection of *quassia*. When it is remembered that quassia may be used by hop growers like other gardeners to combat aphis, and that large quantities are in fact so used in hop gardens in Oregon and California which send large quantities of hops to the British market, it will be obvious that the mere detection of quassia in beer by a very delicate test affords no certain evidence that the brewer has knowingly made use of quassia as such.

In the session of 1910, two bills were introduced in the British House of Commons, one of which proposed to prohibit the use by brewers of any bitter article or preservative other than hops, the other making an exception in favour of sulphur dioxide and sulphites. If either of these bills should ever become law, hop bitter substitutes would entirely disappear from use and the analyst charged with the administration of the Act would direct his attention to the detection of sulphites, salicylic acid, etc., which offer some real advantages to the brewer, and might possibly, in spite of prohibition, be used for conferring stability on the very lightly hopped ales for which there is increasing demand. But to employ a staff of analysts in examining British beers for bitter substances other than hops would be a waste of public money.

ANIMAL BASES.

By A. E. TAYLOR.

The distinction formerly drawn between the plant and animal bases possesses to-day only historic interest; and of historical interest also is the distinction between natural and synthetic bases. The higher orders of plants and the lower orders of plants (bacteria) present synthetic and metabolic processes that are in many respects analogous to the chemical and metabolic processes that are observed in the higher and lower forms of animal life. While some bases are formed only in plants and others are formed only in animals, many are synthesised in both orders, and nearly all may be built up in the laboratory. The relations of bacterial processes to the alimentary tract and to diseases of the higher animals, furthermore, render a classification of bases on the basis of source of derivation very undesirable. In the concrete instance, it may be of the highest importance to determine whether a particular base be of bacterial or metabolic origin. But this affords no basis for a chemical classification. Convenience dictates the arrangement of the bases in the present chapter. In modern organic chemistry, the amino-acids are no longer regarded as bases; nor are the aromatic derivatives of certain amino-acids to be here regarded as bases.

The animal bases of known constitution may nearly all be arranged in one or other of the following groups or classes:

1. *Pyridine Derivatives;* bases specially characteristic of putrefactive change.

2. *Monamines;* including methylamine, trimethylamine, and other similar bases.

3. *Diamines;* including piperazine, and certain bases observed to be produced in the putrefactive decomposition of proteins.

4. *Amino-acids;* including glycocoll, leucine, tyrosine, asparagine, etc.

5. *Betaïnes*, a special class of amino-bases; including betaïne, choline, neurine, muscarine, etc.

6. *Urea* and its analogues and derivatives.

7. *Imino-bases;* including guanidine, glycocyamine, creatine, crea-tinine, histidine, etc.

8. *Purine* or *Alloxuric bases;* including xanthine, hypoxanthine, guanine, adenine, etc.

The most important bases of the first two classes have already been considered in Vol. VI. Further information respecting some of them will be found under "Ptomaïnes," as will also descriptions of numerous bases of which the constitution is not yet known. The diamines of interest which were not described in Vol. VI, are considered below; the bases of classes 4 to 8 are described *seriatim* in the sequel; and the ptomaïnes as a class are considered in a separate section.

DIAMINES.

The diamines are a class of bases derived from a double molecule of ammonia by the replacement of two or more of the hydrogen atoms by hydrocarbons of the olefine, phenylene or naphthylene series. The diamines of phenylene, tolylene, and naphthylene have been de-scribed in Vol. VI. Piperazine (which has the constitution of a diethylene-diamine) and its analogue spermine, are described below, whilst neuridine, cadaverine, and other putrefaction-products having the constitution of diamines are considered under "Ptomaïnes."

Ethylene-diamine.

$$C = H_2 \diagup NH_2$$
$$\vert$$
$$C = H_2 \diagdown NH_2$$
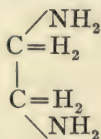
Ethylene-diamine is formed, together with several allied bases, by the reaction of ethylene bromide and alcoholic ammonia at 100°. After cooling, the liquid portion is decanted from the ammonium bromide, evaporated to dryness, and distilled with potassium hydroxide. The distillate is digested with solid potassium hydroxide to absorb water, and the bases separated by fractional distillation. In the manufacture of chloral, a by-product is obtained which can be conveniently used for the preparation of ethylene-diamine. The fraction of this product boiling between 70° and 100° contains ethylene and ethylidene chlo-rides, together with higher substitution-products. On treatment with

alcoholic ammonia at 100–120°, the first two bodies are converted into diamines. When the reaction is complete, the liquid is poured off from the separated ammonium chloride, and the unaltered chlorides distilled off. From the liquid left in the retort, the hydrochloride of ethylene-diamine separates out, and is obtained in silver-white needles after repeated recrystallisation and washing with alcohol.

If the brown mother-liquor be distilled with potassium hydroxide, and the first fraction of the distillate treated with hydrochloric acid, a further crop of crystals of ethylene-diamine hydrochloride will be obtained, while the fractions subsequently distilling contain the higher diamines, triamines, etc.

On distilling the hydrochloride thus prepared with potassium hydroxide, a hydrate of ethylene-diamine is obtained of the composition $C_2H_4(NH_2)_2 + H_2O$; from this the anhydrous base can only be obtained by repeated distillation over sodium. It is a viscous liquid, having a faint ammoniacal odour and burning taste. It boils at 117°, and dissolves easily in water to a strongly alkaline liquid.

Ethylene-diamine occurs with other diamines among the products of putrefactive decomposition of proteïns (see Ptomaïnes).

Diethylene-diamine. Piperazine.

$C_4H_{10}N_2$; *i.e.*,
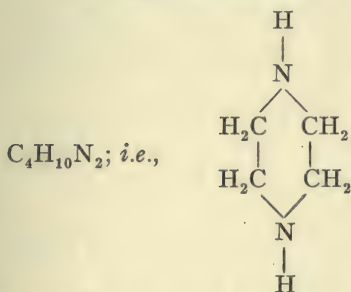
This substance has the constitution of a hexahydropyrazine, standing in the same relation to pyrazine that piperidine does to pyridine. It may be regarded as piperidine in which the CH_2 group in the γ position has been replaced by NH. It has acquired considerable practical interest from its supposed identity with spermine,[1] a

[1] The commercial products known as spermine, piperazine, and piperazidine are stated by A. W. Hofmann to be identical with diethylene-diamine, and Ladenburg admits the probable identity of the base described by him as ethylenamine with diethylene-diamine. Majert and Schmidt have come to the same conclusion (*Ber.*, 23, 3297, 3718, 3740). On the other hand, the natural base originally described by Schreiner under the name of spermine appears to be distinct from diethylene-diamine, and therefore the commercial synthetical product should not be called by the former name.

base occurring in semen and certain human organs; and from the fact of its forming a soluble urate, which character has led to its application as a remedy for gout and rheumatism.

Diethylene-diamine was first obtained by A. W. Hofmann, together with monoethylene-diamine, $(C_2H_4)H_4N_2$, as a product of the reaction of ammonia and ethylene bromide.[1]

Schering has patented the direct production of piperazine by reducing ethylene oxamide by zinc-dust or metallic sodium, according to the equation:

$$\left\{ \begin{matrix} CH_2.NH.CO \\ CH_2.NH.CO \end{matrix} \right\} + 4H_2 = \left\{ \begin{matrix} CH_2.NH.CH_2 \\ CH_2.NH.CH_2 \end{matrix} \right\} + 2H_2O.$$
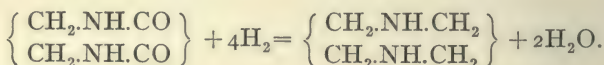
Marckwald and Haltz (*Eng. Patent*, 1892, No. 7120) have protected the manufacture of piperazine from aromatic disulphone-piperazides, a class of substances of the following general formula, in which R represents a phenyl, tolyl, xylyl, or naphthyl residue:

$$R.SO_2.N \left\{ \begin{matrix} CH_2.CH_2 \\ CH_2.CH_2 \end{matrix} \right\} N.SO_2.R$$
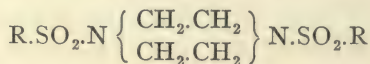
On heating the disulphone-piperazides with dilute acid to 200–250°, or with strong sulphuric acid to 180–200°, they yield piperazine sulphate, from which the free base is obtained by heating with powdered sodium hydroxide.

Piperazine was first obtained in a pure crystallised state by Majert and Schmidt, who prepared it by boiling dinitrosodiphenyl-piperazine with aqueous potassium hydroxide and a little alcohol.[2] It dissolves

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[1] Hofmann has shown (*Ber.*, **23,** 3711) that on hydrolysis of the product formed by the action of ammonia on bromethylene, basic oils are obtained, which on distillation yield fractions of continually rising b. p., the last portions distilling above the range of a mercurial thermometer. On redistillation, the fraction passing over between 117° and 121° yields ethylene-diamine, $(C_2H_4)H_4N_2$. The fraction from 200–225°, on addition of hydrochloric acid, gives a crystalline salt consisting chiefly of the hydrochloride of diethylene-triamine, $(C_2H_4)_2H_5N_3$. The fraction from 250–300° gives, on addition of hydrogen bromide, triethylene-tetramine hydrobromide, $C_6H_{18}N_4.4HBr$. This salt, which is very soluble in water but only slightly so in alcohol, on treatment with potassium hydroxide yields the free base as a colourless viscid liquid of 0.9817 sp. gr. at 15°, which at 18° solidifies to a radiating crystalline mass melting at 12°, and becoming limpid on gentle warming. It dissolves in water with development of much heat, forming a strongly alkaline liquid which absorbs carbon dioxide with avidity. Hofmann has also studied the action of heat on the hydrochlorides of the above bases, the most characteristic product being diethylene-diamine.

[2] This reaction has been patented by W. Majert (*Eng. Patent*, 1890, No. 15, 404), who directs that 1 part of dinitrosodiphenyl-piperazine (or other analogous derivative of piperazine) should be boiled with from 2 to 4 parts of a 25 % solution of sodium or potassium hydroxide, when 1 molecule of piperazine and 2 of nitrosophenol are formed. On distilling the mixture, the former passes over with the steam, and is neutralised in the distillate by hydrochloric, phosphoric, or sulphuric acid. Other modifications of the process are described in English patents, 11,957 of 1891, 4,497 of 1892, and 5,320 of 1893.

According to the second of these patents (B. Willcox), dinitrosodiphenyl-piperazine is converted into piperazine by means of acid sulphites according to the equation:

after a time, when the alcohol is distilled off, more potassium hydroxide added, and the product distilled until only small quantities of liquid pass over. The alkaline distillate is treated with hydrochloric acid, and the resulting piperazine hydrochloride purified by precipitation of its aqueous solution with absolute alcohol. From the hydrochloride the free base is obtained by treatment with alkali hydroxide and distillation.

Piperazine forms well-defined, colourless, four-sided, glittering tables, which melt at 104–107° when heated in capillary tubes; although when the m. p. is determined on larger quantities it is found to be 112°—a difference which is probably due to the extremely hygroscopic nature of the base. Piperazine, as usually met with, boils at about 140° (but at 145° when purified by treatment with sodium) and solidifies on cooling to a hard crystalline mass.

Piperazine has a faint, aromatic odour, is practically tasteless, and is neither poisonous nor caustic. It is extremely deliquescent and soluble in water, from which menstruum it crystallises in glittering quadratic tables. It is deposited from absolute alcohol in large, transparent crystals.

The aqueous solution of piperazine has a strongly alkaline reaction, while the solid substance readily absorbs carbon dioxide from the air and is converted into the carbonate, melting at 162–165°.[1]

Majert and Schmidt have described the following series of *hydrates* of piperazine, that most readily-formed being a hexahydrate, which crystallises from dilute aqueous solutions.

Hydrate	Formula	M. P., ° C.
Monohydrate	$C_4H_{10}N_2 + H_2O$	75
Dihydrate	$C_4H_{10}N_2 + 2H_2O$	56
Trihydrate	$C_4H_{10}N_2 + 3H_2O$	39–40
Tetrahydrate	$C_4H_{10}N_2 + 4H_2O$	42–43
Pentahydrate	$C_4H_{10}N_2 + 5H_2O$	45
Hexahydrate	$C_4H_{10}N_2 + 6H_2O$	48

$$C_6H_4(ON) - N\begin{array}{c}CH_2-CH_2\\CH_2-CH_2\end{array}N.C_6H_4.ON + 6NaHSO_3$$

$$= \left[NH\begin{array}{c}CH_2-CH_2\\CH_2-CH_2\end{array}NH\right],H_2SO_3$$

$$+ 2C_6H_2.(OH)(NH_2)(SO_2.ONa)_2 + Na_2SO_3.$$
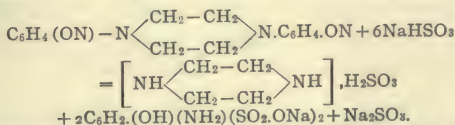
The dinitrosodiphenyl-piperazine is treated with a 40 % aqueous solution of the acid sulphite and the mixture heated to the b. p., when a violent reaction occurs, and the dinitroso-compound is completely dissolved with the formation of a yellow liquid. When the action is ended the solution is acidified with hydrochloric or with sulphuric acid, when a portion of the amino-phenol sulphuric acid separates, and is filtered off. The filtrate is then made alkaline and the piperazine distilled over with steam. It is purified by conversion into the hydrochloride and subsequent decomposition of the latter.

[1] Majert and Schmidt consider that the body regarded by Ladenburg as piperazine was in reality an impure carbonate of the base, as proved by its m. p., 159–163°.

Piperazine hydrochloride forms snow-white matted needles, containing $B(HCl)_2 + H_2O$. It is deliquescent, and very soluble in water, insoluble in alcohol, tastes like ammonium chloride, and is not poisonous. B,H_2PtCl_6 crystallises in small yellow needles, moderately soluble in hot water, but only very sparingly soluble in hot alcohol. B,H_2HgCl_4 crystallises in concentrically grouped needles, readily soluble in hot water, but reprecipitated on adding alcohol. $B,HAuCl_4$ forms small, yellow, glittering scales.

Piperazine is especially characterised by the formation of an insoluble pomegranate-red double salt with bismuth, which is precipitated on adding potassium bismuth iodide to a dilute, slightly acid solution of piperazine hydrochloride. It forms microscopic rods or rectangular plates, insoluble in hydrochloric acid. By this reaction, piperazine may be directly detected in urine containing it.

Piperazine urate, $C_4H_{10}N_2,C_5H_4O_3N_4$, is a salt which dissolves in 50 parts of water at 17° C. Even in presence of a large excess of uric acid, this neutral and soluble salt is formed, and is said to be capable of dissolving a large excess of uric acid.[1] The ready solubility of piperazine urate has led to the employment of piperazine hydrochloride in gout, and rheumatism, in doses ranging from 0.5 to 3.0 grm. daily. Piperazine passes through the human organism unchanged, and may be found in the urine in a very short time.[2] It is best detected by boiling the urine, filtering from albumin or other precipitate, acidifying the filtrate with hydrochloric acid, concentrating it to a small bulk, and again filtering. The filtrate is treated with strong sodium hydroxide solution and distilled. In the distillate the piperazine may be recognised by adding potassio-bismuth iodide, when the characteristic bismuth compound is precipitated in garnet-red microscopic crystals.

Piperazine picrate is precipitated, on adding excess of picric acid to a solution of piperazine, in pale yellow needles, which are insoluble in cold water or in hydrochloric acid and exhibit a characteristic micro-

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[1] How the recorders of this observation distinguished a mixture of free uric acid with the neutral urate from a soluble urate is not evident. What is meant is probably that excess of uric acid does not lead to the formation of an insoluble salt.

[2] Finzelberg has pointed out that in order to ensure the full exercise of the power of piperazine to dissolve uric acid, its retention in the blood for some time should be aimed at. Hence the base is preferably given in solution, and not in pills or powders. As piperazine does not produce irritation of the mucous membrane of the bladder, even in 3 to 5 % solution, it may be injected locally for breaking down urinary calculi of mixed composition. J. Fawcett finds that an aqueous solution of piperazine dissolves uric acid calculi, but a solution of it in urine of the strength of 1 in 1,000, which is above that usually found in the urine after taking the drug internally, has no effect whatever (*Brit. Med. Jour.*, 1894, **2**, 426).

scopic appearance.   The salt is soluble in hot water or in a solution of piperazine.

Biesenthal found in two cases that the addition of picric acid to the urine of persons who had taken piperazine produced precipitates which he at first regarded as due to albumin; but he subsequently concluded that they were produced by piperazine itself, which passes unchanged through the system, and gives a precipitate with picric acid even when dissolved in 20,000 parts of water.   Piperazine can thus be detected in the urine three or four hours after it has been taken.   The picrate has a characteristic crystalline form, and cannot be mistaken for the amorphous precipitate produced by albumin.   Further, the precipitate of piperazine picrate dissolves on heating, and reappears on cooling, while that due to albumin is permanent.   The nature of the precipitate may be proved by treating it with hydrochloric acid, removing the picric acid by extraction with ether, and recognising the piperazine by its reaction with bismuth potassio-iodide.

Dinitroso-piperazine, $C_4H_8N_2(ON)_2$, is obtained when sodium nitrite is added to a solution of piperazine hydrochloride containing free hydrochloric acid, and the mixture warmed for a short time.   A crystalline substance separates out, which, when purified by crystallisation from boiling water, forms yellowish lustrous plates, melting at 158° and sparingly soluble in cold water or ether, but readily in boiling water or hot ether.   Dinitroso-piperazine is not decomposed by boiling with alkali hydroxides, or by cold concentrated hydrochloric or sulphuric acid.   It gives a deep blue colouration, after some minutes, with a solution of phenol in concentrated sulphuric acid (Liebermann's reagent).

The quantitative estimation of piperazine may be undertaken either by the formation of the salt with platinic chloride, recrystallised from hot alcohol, or by the formation of the picrate, recrystallised from hot water, and weighing.

Ethylene-ethenyl-diamine, $\begin{array}{c} CH_2.NH \\ | \qquad\qquad \Large\rangle C.CH_3, \\ CH_2{-}N \end{array}$ has been proposed,

under the name of "*lysidine*," as a substitute for piperazine in gout. Lysidine is a very hygroscopic, reddish-white, crystalline substance, having a peculiar odour resembling coniine.   It is easily soluble, and occurs commercially as a 50% aqueous solution, from 2 to 10 grm. of which are directed to be taken at a time in aërated water.

Spermine is a base occurring as a crystalline phosphate in the secretions and certain organs of animals. This phosphate, sometimes known as "Charcot's crystals," appears to be especially plentiful in the spleen, liver, and blood of men and animals suffering from leucocythæmia,[1] as also in the expectorations in cases of bronchial asthma. They are also found on the surface of the spirit used for preserving pathological preparations, and have been mistaken for tyrosine, calcium phosphate, and other compounds. Later researches have shown that the crystals occur in various healthy tissues, but are most characteristic of semen, of which they form about 5 % of the solid constituents. They were prepared by P. Schreiner (*Annalen*, **194,** 68) from fresh human semen by boiling it with alcohol, separating and drying the precipitate, treating it with warm water rendered alkaline with ammonia, and concentrating the filtered solution. The crystals which separate are purified by recrystallisation from hot water containing a little ammonia. From the spermine phosphate thus prepared the free base is obtained by boiling with baryta-water, and evaporating the filtered solution.

Spermine is a colourless, odourless, crystalline substance, soluble in water and alcohol to form strongly alkaline solutions.

Spermine phosphate is immediately precipitated as crystals on adding phosphoric acid to an alcoholic or aqueous solution of free spermine. This reaction distinguishes true spermine from commercial piperazine. The crystals, which are chiefly prisms and stellate aggregates of acutely rounded pyramids, have a characteristic microscopic appearance. They are colourless, brittle, slightly soluble in cold but readily in hot water, and in dilute acid and alkaline liquids; but are insoluble in alcohol, ether, or chloroform. Spermine phosphate contains two atoms of nitrogen to one of phosphorus, loses 3 H_2O. at 100°, melts at 170°, and at a higher temperature decomposes with evolution of ammonia.

Spermine hydrochloride is crystalline; the platinichloride forms large prismatic crystals. Auric chloride precipitates from solutions of spermine hydrochloride the *aurichloride*, which crystallises in goldenyellow plates, soluble in ether, alcohol, and water. When an aqueous

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[1] Spermine is extracted from these sources by boiling with water containing acetic acid, precipitating the solution with lead acetate, removing the excess of lead by hydrogen sulphide, and precipitating the base by phosphotungstic acid. Free spermine is obtained by boiling the precipitate with barium hydroxide-water, and evaporating the filtered solution.

solution of this salt is treated with metallic magnesium, an odour resembling that of fresh human semen is evolved.[1]

Spermine bismutho-iodide crystallises in long, pointed needles, often united to form feathery aggregates. The microscopic appearance of this salt affords one of the few tangible distinctions between spermine and piperazine (page 200).

The formula attributed to spermine by Schreiner was C_2H_5N, while Ladenburg and Abel suggested that it was identical with diethylene-imine, $C_4H_{10}N_2$, which has the same percentage composition. This again is very probably the same substance as piperazine. Pöhl, however, has prepared spermine by a method similar to that of Schreiner, and finds that its properties fully agree with his description; but, on the other hand, the analysis of the platinichloride gave numbers which did not agree with the formula C_2H_5N, but with the composition $C_{10}H_{26}N_4$, and the analysis of the aurichloride confirmed this result. Hence, if these results be correct, spermine can be neither identical with nor an isomer of piperazine.

A. Jürgens (*Chem. Central.*, 1891, i, 193; *Pharm. Zeit. Russ.*, **29,** 726) has compared Schreiner's base with that of Pöhl. He confirms the former chemist's results, and states that certain of Pöhl's specimens contained no spermine, since ammonium phosphate and ammonia gave no crystalline precipitate, auric chloride precipitated an amorphous substance, and platinic chloride gave cubical, not prismatic, crystals.

A direct comparison by Majert and Schmidt of piperazine with Schreiner's spermine has shown that these two compounds are not identical, great differences existing between the phosphates and bismutho-iodides of the two bases (*Ber.*, 1891, **24,** 241).

Amino Compounds.

The substituted ammonias, or the amino compounds, comprise for the purposes of the present consideration, four classes of compounds. Amino-acids, fatty acids in which the hydrogen of the methyl or methylene groups is replaced by the NH_2 group; amides, ammonia in which the hydrogen is replaced by acid radicles, or the hydroxyl of fatty acids is replaced by aminogen; amines, ammonia in which the hydrogen is

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[1] A. Jürgens considers that this reaction has only a negative value, since the filtrate from spermine phosphate gave the same smell when similarly treated; but it is possible that the trace of spermine remaining in solution sufficed to produce the odour.

replaced by alkyl radicles; and alkylamides, ammonia in which the hydrogen is replaced partly by acid and partly by alkyl radicles. A typical example of amino-acid is glycocoll, $\begin{matrix} CH_2NH_2 \\ | \\ COOH \end{matrix}$. An example of an amide is acetamide $\begin{matrix} CH_3 \\ | \\ CONH_2 \end{matrix}$.

By the replacement of the hydrogen of ammonia by acid-radicles, or by the replacement of the hydroxyl-group of acids by NH_2, amides are produced which may be primary, secondary, or tertiary, according to the number of hydrogen-atoms or hydroxyl-groups thus substituted. Thus there may be obtained from acetic acid, $CH_3.COOH$:

Acetamide,	$(C_2H_3O).NH_2$
Diacetamide,	$(C_2H_3O)_2.NH$
Triacetamide,	$(C_2H_3O)_3.N$

Alkylamides are compounds derived from ammonia by the simultaneous replacement of its hydrogen by acid and alkyl-radicle, as in ethyl-acetamide or acetyl-ethylamine:

$$NH\begin{cases} C_2H_5 \\ C_2H_3O \end{cases}$$
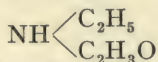
Acetanilide (Vol. VI.), $C_6H_5.NH.C_2H_3O$, is a substance of the same class.

The amides are obtained:

1. By heating the ammonium salts of the corresponding acids to about 230°: $C_2H_3O.ONH_4 = H_2O + C_2H_3O.NH_2$.

2. By the addition of the elements of water to the cyanide of the next lower alkyl-radicle: $CH_3.CN + H_2O = CH_3.CO.NH_2$.

This assimilation of water is frequently effected by dissolving the nitrile in concentrated sulphuric acid, or in a mixture of acetic and sulphuric acids; or by the action of cold concentrated hydrochloric acid. Also, and often quantitatively, by treatment with hydrogen peroxide.

3. By the action of ammonia on acid chlorides or anhydrides:

a. $CH_3CO.Cl + 2NH_3 = CH_3CO.NH_2 + NH_4Cl.$

b. $[(CH_3CO)O]_2 + 2NH_3 = CH_3CO.NH_2 + C_2H_3O.ONH_4.$
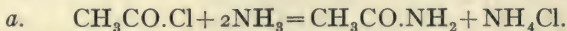
4. By the reaction of ethereal salts with ammonia, the change sometimes occurring in the cold:

$$CH_3CO.OC_2H_5 + NH_3 = CH_3CO.NH_2 + H.OC_2H_5.$$
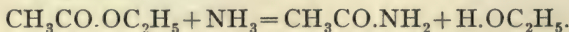
5. The secondary and tertiary amides result from the treatment of the acids or anhydrides with their nitriles:

$$a.\ CH_3.CN + CH_3.CO.OH = (CH_3CO)_2NH.$$
$$b.\ CH_3.CN + (CH_3CO)_2O = (CH_3CO)_3N.$$
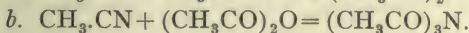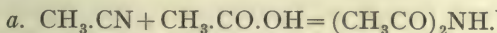
The foregoing reactions are those of acetamide, which is the typical substances of the series.

The amides are readily saponifiable. When they contain both acid and alkyl-radicles only the former is saponified:

$$C_2H_3O.NH(C_2H_5) + H.ONa = C_2H_3O.ONa + H.NH(C_2H_5).$$

Ethyl-acetamide. Sodium acetate. Ethylamine.
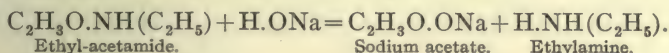
Nitrous acid acts on the amides with formation of the corresponding acids and liberation of nitrogen. The reaction consists in an exchange of a hydroxyl-group for an amino-group (OH for NH_2):

$$C_2H_3O.NH_2 + NO.OH = C_2H_3O.OH + H_2O + N_2.$$
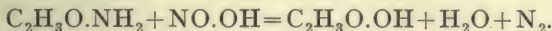
This reaction may be applied to the estimation of amino-compounds (see also Vol. VI).

When treated with bromine and alkali the primary amides form bromo-derivatives, which are converted by further treatment with alkali into amines thus:

$$C_2H_3O.NH_2 + Br_2 = C_2H_3O.NHBr + HBr.$$
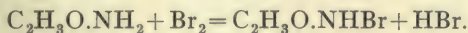
These bromo-derivatives react with more amide and alkali to form peculiar substituted ureas, e.g., methyl-acetyl-urea, $NH(CH_3).CO.NH(C_2H_3O)$, which are split up by further alkali with formation of amines containing one carbon-atom less than the original amide. This reaction affords an excellent means of preparing the lower amines (see Methylamine, Vol. VI); but in the case of amides containing more than five carbon-atoms the reaction is less productive of amines, as it is complicated by the formation of nitriles and lower acids through the action of the excess of bromine on the amines produced:

$$C_nH_{2n+1}.CH_2.NBr_2 = C_nH_{2n+1}.CN + 2HBr;\ and,$$
$$C_nH_{2n+1}.CN + 2H_2O = C_nH_{2n+1}.COOH + NH_3.$$
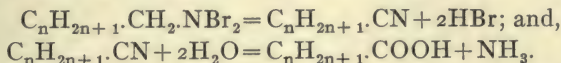
A modified reaction occurs when amides are treated at once with a solution of sodium hypobromite containing an excess of sodium hydroxide. Under these circumstances the nitrogen is in some cases wholly or partly evolved as gas.

The amides, though derivatives of ammonia, have very feebly-

marked basic characters. The primary amides are, however, capable of forming hydrochlorides [*e.g.*, acetamide hydrochloride, $(C_2H_3O.NH_2)_2HCl$] and certain other salts, but such compounds are very unstable, being decomposed in most cases by water alone (compare acetanilide). On the other hand, the hydrogen of the NH_2-group can be replaced by metals, especially mercury, the amide existing as an acid in such compounds.

The amides are remarkable for their high m. p. and b. p. as compared with the corresponding amines. Thus acetamide, $C_2H_3O.NH_2$, does not *melt* below 222°, whereas ethylamine, $C_2H_5.NH_2$, *boils* at 19°.

By the exchange of the oxygen in amines for the imino-residue, NH, *amidines* or imino-amines are formed, of which class the typical substance is acetamidine: $CH_3.C(NH).NH_2$.

The amidines have well-characterised basic properties, but are very easily saponified by boiling either with alkalies or with acids.

Amino-acids are compounds in which NH_2 replaces an atom of the hydrogen of the alkyl-group, whereas in the amines the OH of the *carboxyl*-group is replaced, as is shown by the following formulæ:

$$\begin{array}{ccc} CH_3 & CH_2NH_2 & CH_3 \\ | & | & | \\ COOH & COOH & CO.NH_2 \end{array}$$

Acetic acid.   Amino-acetic acid.   Acetamide.
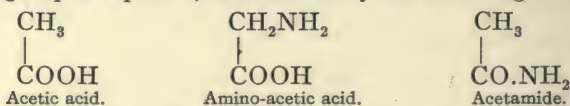
The amino-acids result from the replacement of one of the hydrogen-atoms in direct union with carbon by the amino-group NH_2, which can be effected, among other methods, by treating the chloro- or bromo-acid with ammonia:

$$CH_2Br.COOH + NH_3 = HBr + CH_2(NH_2).COOH.$$

Bromacetic acid.   Amino-acetic acid.
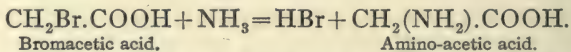
On the other hand, when the *hydroxyl* of the acid is replaced by an amino-group an acid amide is formed:

$$CH_3.CO.Cl + NH_3 = HCl + CH_3.CO(NH_2).$$

Acetyl chloride.   Acetamide.
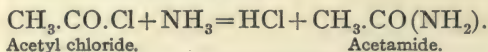
Amic acids are derivatives of polybasic acids intermediate between the amino-acids and amides. They contain both an amino-group, NH_2, and a carboxyl-group, COOH, whereas the latter is absent from the corresponding amides.

By treating amino-acids and amic acids with nitrous acid, the corresponding hydroxy-acids are formed and nitrogen evolved. Thus, aspartic acid (succinamic acid) yields hydroxy-succinic acid (malic

acid) by reaction with nitrous acid. Amino-acetic acid (glycocoll) yields hydroxy-acetic acid (glycollic acid).

The table on the preceding page shows the formulæ and relationships of some of the more important substances of the amino-group. Among other interesting synthetical compounds of analogous constitution are:

Acetyl-glycocoll, *Aceturic acid*,
$$CH_2.NH(C_2H_3O)$$
$$|$$
$$COOH$$

Ethyl carbamate, *Urethane*,
$$C=O \begin{cases} NH_2 \\ O(C_2H_5) \end{cases}$$

Phenyl-urethane, *Euphorin*,
$$C=O \begin{cases} NH.(C_6H_5) \\ O(C_2H_5) \end{cases}$$

Glycocoll. Glycine. Amino-acetic acid.
$$C_2H_5NO_2; \quad \begin{matrix} CH_2NH_2 \\ | \\ COOH \end{matrix}$$

Glycocoll does not appear to occur frequently ready-formed in nature, though it is said to occur in the muscle of *Pecten irradians*. Glycocoll is a very frequent product of the action of acids or alkalies on animal matters. Thus it was first obtained by Braconnot in 1820 by boiling glue with sulphuric acid, whence its name of glycocoll or sugar of gelatin. It likewise results from the action of alkali hydroxides on meat or gelatin, and is also formed when hippuric acid is boiled with hydrochloric acid, or when glycocholic or hyoglycocholic acid is boiled with barium hydroxide water.

$$(C_7H_5O).NH.CH_2.COOH + OH_2 = C_7H_5O.OH + NH_2.CH_2.COOH$$
Hippuric acid.   Benzoic acid.   Glycocoll.

$$(C_{24}H_{39}O_4).NH.CH_2.COOH + OH_2 = C_{24}H_{40}O_5 + NH_2.CH_2.COOH$$
Glycocholic acid.   Cholic acid.   Glycocoll.
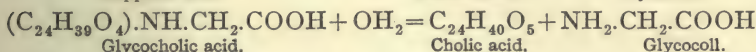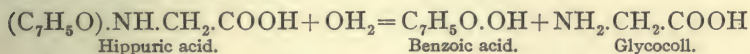
Glycocoll is best prepared by boiling hippuric acid for half an hour with 4 parts of fuming hydrochloric acid. The product is diluted with water and allowed to cool, when the greater part of the benzoic acid crystallises out. The remainder is removed by extraction with ether or petroleum-spirit, and the solution of glycocoll hydrochloride evaporated till the salt crystallises on cooling. It is washed with absolute alcohol, and on treatment with an equivalent amount of litharge or oxide of silver yields free glycocoll, which is recrystallised from water or dilute spirit.

Acid	Amino-acid (H replaced by NH₂)	Amic acid (OH replaced by NH₂)	Amide (CO.OH replaced by CO.NH₂)
CH₃ COOH *Acetic acid.*	CH₂NH₂ COOH Aminoacetic acid. *Glycocoll.*	. . . . . .	CH₃ CO.NH₂ *Acetamide.*
CH₂OH COOH Hydroxyacetic acid. *Glycollic acid.*	CH(NH₂)OH COOH Aminoglycollic acid.	CH₂.NH₂ COOH Glycolamic acid. *Glycocoll.*	CH₂.OH CO.NH₂ *Glycolylamide.*
CH₃ CHOH COOH Hydroxypropionic acid. *Lactic acid.*	CH₂.OH CH.NH₂ COOH Aminolactic acid. *Serine.*	CH₃ CH.NH₂ COOH *Alanine.*	CH₃ CHOH CO.NH₂ *Lactamide.*
$C=O$ /OH \OH Carbonic acid.	. . . . . .	$C=O$ /NH₂ \OH Carbamic acid.	$C=$ /NH₂ \NH₂ Carbamide. *Urea.*
COOH COOH Oxalic acid.	. . . . . .	CO.NH₂ COOH Oxamic acid.	CO.NH₂ CO.NH₂ *Oxamide.*

Acid	Amino-acid (H replaced by NH₂)	Amic acid (OH replaced by NH₂)	Amide (CO.OH replaced by CO.NH₂)
$COOH$ CH_2 CH_2 $COOH$ Succinic acid.	$COOH$ CH_2 $CH.NH_2$ $COOH$ Aminosuccinic acid. *Aspartic acid.*	$CO.NH_2$ CH_2 $CH.NH_2$ $COOH$ Aminosuccinamic acid. *Asparagine.*	$CO.NH_2$ CH_2 CH_2 $CO.NH_2$ Succinamide.
$COOH$ CH_2 $CHOH$ $COOH$ Malic acid.	......	......	$CO.NH_2$ CH_2 $CHOH$ $CO.NH_2$ Malamide.
$COOH$ $CHOH$ $CHOH$ $COOH$ Tartaric acid.	......	$COOH$ $CHOH$ $CHOH$ $CO.NH_2$ Tartramic acid.	$CO.NH_2$ $CHOH$ $CHOH$ $CO.NH_2$ Tartramide.

Glycocoll may be prepared by boiling glycocholic acid for twelve hours with strong hydrochloric acid, filtering from the resinous mixture of cholic acid and dyslysin, and evaporating the filtrate. The glycocoll hydrochloride is dissolved in water and treated with lead hydroxide, the liquid filtered, and the soluble lead compound of glycocoll decomposed by hydrogen sulphide. On concentrating the filtered liquid, glycocoll is deposited in crystals.

Glycocoll may also be prepared by boiling a concentrated solution of chloracetic or bromacetic acid with a large excess of strong ammonia.

Glycocoll has the constitution of an aminoacetic acid, and is the type of the class of substances of which the proteins are built up.

Fig. 6.—Glycocoll.

Glycocoll forms very hard, flattened prisms or aggregated plates, belonging to the monoclinic system (Fig. 6).[1] The crystals grate between the teeth and have a sweet taste, but are not poisonous. When heated to 170°, glycocoll melts, and at a higher temperature decomposes with separation of carbon.

Glycocoll dissolves in about 400 parts of cold and a smaller quantity of boiling water. It crystallises readily by spontaneous evaporation of its aqueous solution. It is moderately soluble in rectified spirit, but insoluble in absolute alcohol, even when boiling, as also in ether. Glycocoll is optically inactive. It is not susceptible of alcoholic fermentation.

When glycocoll is boiled with *concentrated* alkali hydroxide it evolves ammonia, and on treating the residue with hydrochloric acid hydrocyanic acid is disengaged, while oxalic acid is found in the liquid.

Glycocoll is charred by strong sulphuric acid. Distilled with dilute sulphuric acid and manganese or lead dioxide, it yields hydrocyanic and carbonic acids:

$$C_2H_5O_2N + O_2 = 2H_2O + CO_2 + HCN$$
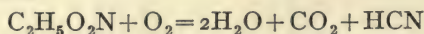
Nitrous acid converts glycocoll into glycollic acid, thus:

$$CH_2(NH_2).COOH + HNO_2 = CH_2(OH).COOH + H_2O + N_2$$
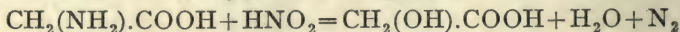
On agitating the liquid with ether, the glycollic acid is dissolved.[2]

[1] Mere traces of impurities influence the crystalline form and other physical characters of glycocoll in a remarkable manner.

[2] On separating and evaporating the solution, the glycollic acid is obtained in fine laminæ, which are unchanged in the air, melt at 80°, and are readily soluble in alcohol.

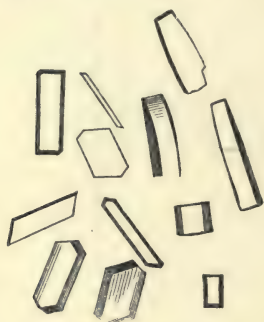Glycollic acid. $CH_2(OH).COOH$, forms crystallisable salts, most of which are readily

Glycocoll evolves no nitrogen when treated with an alkaline solution of sodium hypobromite (A. H. Allen).

On addition of mercurous nitrate, cold solutions of glycocoll yield a grey precipitate of metallic mercury, but the reaction occurs more readily on heating.

On addition of ferric chloride to a solution of glycocoll, a strong red colouration is produced. This is destroyed by acids, but reappears on cautious neutralisation. Hence, aminoacetic acid reacts with ferric salts much like acetic acid itself.

When treated with a drop of phenol and then with a solution of sodium hypochlorite, glycocoll gives a blue colouration, in this reaction resembling ammonia, aniline, and methylamine.

On heating glycocoll in a sealed tube with benzoic acid, hippuric acid is produced. The same product is formed by treating glycocoll with hydrochloric acid and benzoyl chloride. This reaction has been proposed by C. S. Fischer (*Zeit. physiol. Chem.*, **29,** 164) for the determination of glycocoll, and the value of the method has been confirmed by M. Gonnermann (*Pflüger's Archiv*, 1894, **59,** 42). When glycocoll is taken internally, it appears in the urine as hippuric acid, which has the constitution of a benzoyl-glycocoll, $(C_6H_5.CO).NH.CH_2.COOH$.

Glycocoll is neutral to litmus, but has at once the characters of an acid and a base (amphoteric.) It combines with metallic oxides, and forms crystallisable salts with acids. On boiling an aqueous solution of glycocoll with cupric hydroxide or acetate, cupric aminoacetate, $Cu(C_2H_4O_2N)_2 + H_2O$, separates in fine blue needles on cooling or on adding alcohol. The compound dissolves in alkali hydroxide with deep blue colour. If glycocoll and potassium hydroxide be added to a solution of cupric sulphate, the liquid becomes dark blue, and the above salt is precipitated on adding alcohol.

The barium, strontium, calcium, and magnesium salts of aminoacetic acid have been obtained in a crystallised state (*Annalen*, **266,** 292), and the mercury, lead, cadmium, and palladium salts are likewise crystalline. The silver salt crystallises in tablets, has a strong alkaline reaction, turns grey in the light, and decomposes at 100°.

In addition to the homologues, several important and interesting derivatives of glycocoll occur naturally in the animal kingdom. Among

soluble. The neutral *lead* salt is soluble in cold water, but on boiling its solution, or on precipitating a glycollate with lead acetate, a basic salt, $(C_2H_3O_3Pb)_2O$, separates in stellar, needles, requiring 10,000 parts of water for solution. *Cupric glycollate*, $Cu(C_2H_3O_3)_2$, forms blue crystals, which require 134 parts of cold water for solution.

these substances are hippuric and salicyluric acids, occurring in urine; glycocholic acid, a constituent of bile; glycocyamine, etc.

Glycocoll hydrochloride, $C_2H_5O_2N,HCl$, forms deliquescent crystals, having an acid reaction and astringent taste. It is readily soluble in water, but only slightly in alcohol. B_2,HCl forms trimetric crystals. B_2,H_2SO_4 forms large non-deliquescent prisms, soluble in water, but insoluble in alcohol or ether. B,HNO_3 and $B_2,H_2C_2O_4$ are also crystallisable.

In consequence of the double function exerted by the amphoteric glycocoll, it not only combines both with bases and acids, but forms a peculiar class of compounds with neutral salts. Of these the type is the potassium nitrate compound: $NO_3.NH_3.CH_2.COOK$.

The copper salt of glycocoll crystallises in fine blue needles. The crystals of the hydrochloride of the ethyl ester melt at 144°. This is the best state in which to isolate glycocoll, and a quantitative estimation may be grounded upon the nitrogen estimation of this salt. The compound with β-naphthalene-sulpho-chloride melts at 159°, the phenyl-isocyanate melts at 195°. The calcium salt of glycocoll-carbamic acid is also characteristic.

Glycocoll is the lowest member in the series of amino-acids, now recognised as among the most important components of the body. The proteins are composed of amino-acids, condensed with the extrusion of water. When the proteins are hydrolysed, water is added and the amino-acids set free. These amino-acids are fatty acids in which a hydrogen in the methyl or methylene group is replaced by NH_2. We have amino-acids and diamino-acids; we have also amino-acids containing two carboxylic groups. The amino-acids are all of one type, the hydrogen replaced is attached to the α-carbon, the first one above the carboxylic group. In the case of the diamino-acids, there is no rule for the location of the second NH_2 group. The amino-acids that contain one amino and one carboxylic group are typically amphoteric. Those that contain a second COOH group are quite acid. The diamino-acids are quite strongly basic. All amino-acids above glycocoll contain an asymmetric atom of carbon (the one to which the NH_2 is attached) and thus rotate the plane of polarised light. In nature only one optical form usually occurs, the *d* or *l* form as the case may be. But the opposite form can always be prepared in the laboratory; and the racemic amino-acids may thus be prepared. The optical rotation is an important analytical property of these substances.
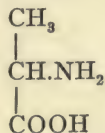
The power of proteins to rotate the plane of polarised light is of course the expression of the property in the component amino-acids, just as the amphoteric, acid or basic property of native proteins is the expression of the NH_2 and COOH groups of the amino-acids. When amino-acids are combined to form proteins, it is through a linkage in which the COOH group of one amino-acid is linked to the NH_2 group of another amino-acid.

There are certain chemical reactions that are common to all the members of the group of amino-acids. These will be first detailed. Then the methods for the isolation and separation of the amino-acids will be described, following which the properties and relations of the individual members of the group will be stated. The isolation of amino-acids may be necessary in the study of digestion mixtures, of the urine and fæces, in the study of germination in plant seeds and in general in investigations into the protein metabolism of animals and plants. It is also now possible to separate different proteins by the study of their content of the different amino-acids. The following are the important amino-acids that are known to occur in the different native proteins, with their formulæ.

Glycocoll, amino-acetic acid,

$$\begin{array}{c} CH_2NH_2 \\ | \\ COOH \end{array}$$

Alanine, α-amino-propionic acid,

$$\begin{array}{c} CH_3 \\ | \\ CH.NH_2 \\ | \\ COOH \end{array}$$

Serine, α-amino-β-hydroxypropionic acid,

$$\begin{array}{c} CH_2.OH \\ | \\ CH.NH_2 \\ | \\ COOH \end{array}$$
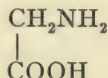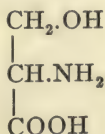
Cystin: This is a double molecule of cystein. Cystein is α-amino-β-thio-propionic acid,

$$
\begin{array}{l}
CH_2.SH \\
| \\
CH.NH_2 \\
| \\
COOH
\end{array}
$$
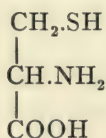
Cystin has therefore the formula
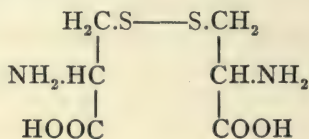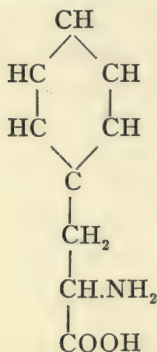
$$
\begin{array}{cc}
H_2C.S \!\!\!-\!\!\!-\!\!\! S.CH_2 \\
| \qquad\qquad | \\
NH_2.HC \qquad CH.NH_2 \\
| \qquad\qquad | \\
HOOC \qquad COOH
\end{array}
$$

Phenylalanine, Phenyl-α-amino-propionic acid,

$$
\begin{array}{c}
CH \\
HC \quad CH \\
HC \quad CH \\
C \\
| \\
CH_2 \\
| \\
CH.NH_2 \\
| \\
COOH
\end{array}
$$

Tyrosine, hydroxy-phenyl-α-amino-propionic acid,

$$
\begin{array}{c}
C.OH \\
HC \quad CH \\
HC \quad CH \\
C \\
| \\
CH_2 \\
| \\
CH.NH_2 \\
| \\
COOH
\end{array}
$$

Tryptophane, Indole-α-amino-propionic acid,

$$
\begin{array}{ccccc}
& \text{CH} & & & \\
& \diagup\diagdown & & & \\
\text{HC} & \text{C}\!\!-\!\!-\!\!\text{C}\!\!-\!\!-\!\!\text{CH}_2 & \\
| & | & | & | \\
\text{HC} & \text{C} & \text{CH} & \text{CH.NH}_2 \\
\diagdown\diagup & & \diagdown\diagup & | \\
\text{CH} & \text{NH} & & \text{COOH}
\end{array}
$$

Histidine: This is α-amino-β-iminazolyl-propionic acid,

$$
\begin{array}{c}
\text{CH}\!\!-\!\!\text{NH} \\
\| \qquad\quad \diagdown \\
\qquad\qquad \text{CH} \\
\| \qquad\quad \diagup \\
\text{C}\!\!-\!\!-\!\!\text{N} \\
| \\
\text{CH}_2 \\
| \\
\text{CH.NH}_2 \\
| \\
\text{COOH}
\end{array}
$$

Aspartic acid, α-amino-succinic acid,

$$
\begin{array}{c}
\text{COOH} \\
| \\
\text{CH}_2 \\
| \\
\text{CH.NH}_2 \\
| \\
\text{COOH}
\end{array}
$$

Glutamic acid, α-amino-glutaric acid,

$$
\begin{array}{c}
\text{COOH} \\
| \\
\text{CH}_2 \\
| \\
\text{CH}_2 \\
| \\
\text{CH.NH}_2 \\
| \\
\text{COOH}
\end{array}
$$

Valine, α-amino-isovaleric acid,

$$
\begin{array}{c}
\text{CH}_3 \quad \text{CH}_3 \\
\diagdown\diagup \\
\text{CH} \\
| \\
\text{CH.NH}_2 \\
| \\
\text{COOH}
\end{array}
$$

Ornithine, α-δ-diamino-valeric acid,

$$CH_2.NH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH.NH_2$$
$$|$$
$$COOH$$
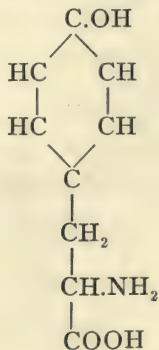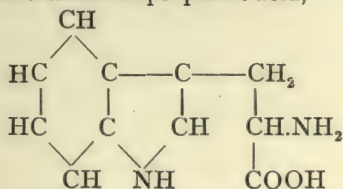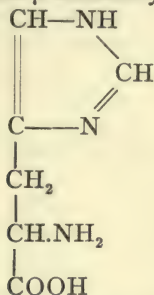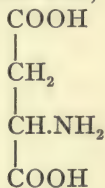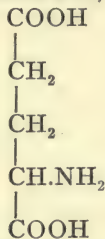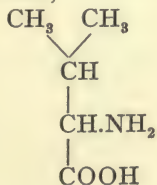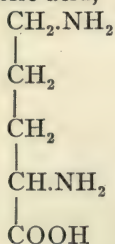
Ornithine does not exist preformed, but only combined with urea to form arginine:

$$NH_2$$
$$|$$
$$HN=C-NH$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH.NH_2$$
$$|$$
$$COOH$$

Leucine, α-amino-isobutyl-acetic acid,

$$CH_3 \quad CH_3$$
$$\diagdown \diagup$$
$$CH$$
$$|$$
$$CH_2$$
$$|$$
$$CH.NH_2$$
$$|$$
$$COOH$$

Lysine, α-ε-diamino-caproic acid,

$$CH_2.NH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH.NH_2$$
$$|$$
$$COOH$$
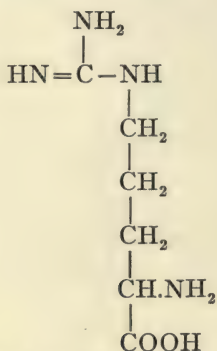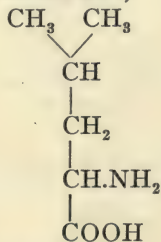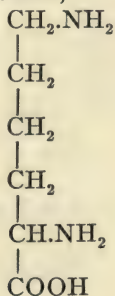
Proline: This is a heterocyclic amino-acid, α-pyrrolidine-carboxylic acid,

$$\begin{array}{cc} CH_2\!\!-\!\!CH_2 \\ | \quad\ | \\ CH_2 \quad CH.COOH \\ \diagdown\!\!\diagup \\ NH \end{array}$$
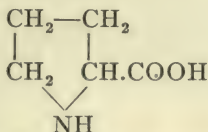
Certain reactions are common to these amino-acids. They combine with alcohols to form esters that are bases. Thus

$$\begin{array}{c} CH_2.NH_2 \\ | \\ COOH \end{array} + C_2H_5OH = \begin{array}{c} CH_2.NH_2 \\ | \\ CO.O.C_2H_5 \end{array} + H_2O$$
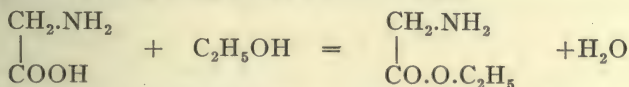
They combine with aldehydes to form methylene compounds that are acids. Thus

$$\begin{array}{c} CH_2.NH_2 \\ | \\ COOH \end{array} + HCOH = \begin{array}{c} CH_2.NCH_2 \\ | \\ COOH \end{array} + H_2O$$

When carbon dioxide is fixed to the amino-group carbamino acids are formed.

$$\begin{array}{c} CH_2.NH_2 \\ | \\ COOH \end{array} + CO_2 = \begin{array}{c} \qquad\ \ H \\ CH_2.N\!\!\diagup \\ | \quad \diagdown COOH \\ COOH \end{array}$$
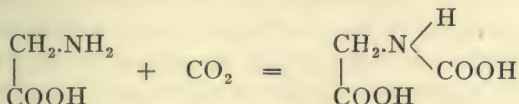
Amino-acids, and oxyamino-acids and peptides as well, combine with β-naphthalene-sulpho-chloride to form insoluble compounds with definite crystallographic properties and sharp m. p. The chlorine is replaced, and the amino-acid is attached to the SO_2 by the amino-group.

$$SO_2\!\!-\!\!NH.CH_2.CO_2H$$
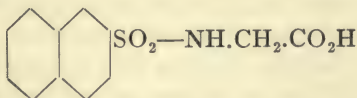
Amino-acids combine with phenyl-isocyanate, to form characteristic crystalline compounds. This may be illustrated for serine.

$$\begin{array}{ccc} CH & & CH_2OH \\ \diagup\!\!\diagdown & & | \\ HC \quad C\!\!-\!\! NH.CO\!\!-\!\!HN.CH \\ | \quad | & & | \\ HC \quad CH & & COOH \\ \diagdown\!\!\diagup & & \\ CH & & \end{array}$$
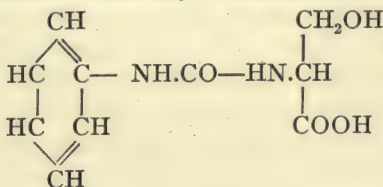
With α-naphthyl-isocyanate amino-acids combine to form well-defined crystalline substances.

$$\text{NH.CO.NH.CH}_2\text{.CO}_2\text{H}$$

Benzoyl chloride yields with amino-acids stable compounds, the chlorine being replaced.

$$
\begin{array}{cc}
\text{CH} & \text{CH}_3 \\
\text{HC} \quad \text{C.CO—HN.CH} \\
\text{HC} \quad \text{CH} \qquad \text{COOH} \\
\text{CH} &
\end{array}
$$
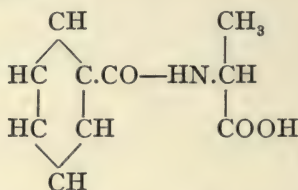
Picrolonic acid forms especially with diamino-acids and diamines well-defined compounds, the mode of attachment being not yet clear.

SEPARATION OF AMINO-ACIDS

The problem of the estimation of amino-acids may arise under one of two circumstances. A material, such as intestinal contents, urine, a bacterial culture medium or autopsy organs, may require analysis for the presence of preformed amino-acids. On the other hand, one may wish to determine the component amino-acids of a protein. In the first instance one proceeds directly with the isolation of the amino-acids. In the second, one must first submit the material to hydrolysis in order to set free the amino-acids, after which one proceeds as before. There are two methods of isolating the amino-acids: the first, by converting them into esters that are separated by fractional distillation; the second, by the formation of insoluble derivatives. The certain method is to practice esterification, and then after separation to distinguish the several amino-acids by means of some particular salt or derivative. The methods to be described originated with E. Fischer.

The hydrolysis may be accomplished by either acids, alkalies or proteolytic ferment. Usually the use of mineral acid is in every way to be preferred. But if tryptophane is to be sought for, the hydrolysis must be done by trypsin. If the material be abundant, it is best to carry through a special search for tryptophane, and this will be described under that heading.

The material to be hydrolysed is suspended in hydrochloric acid of sp. gr .1.19, using 3 parts of the acid to one of the material to be analysed. The acid is then heated until the material has passed into solution, additional acid being added if needed. Under a reflux condenser, the solution is then boiled for some 8 hours. After the solution has cooled, insoluble humin is filtered off, and the precipitate washed until the wash water is clear. The clear solution is then evaporated to a small volume on a water-bath under diminished pressure. The syrup is then placed on ice for several days, in order to give the hydrochloride of glutamic acid opportunity to crystallise out. If such crystallisation occurs, it is removed by filtration and washed with dilute hydrochloric acid and further treated as described under that heading. The combined filtrates are then evaporated to a small volume on the water bath, under diminished pressure, to remove as much hydrochloric acid as possible. The residue is taken up in water and the process repeated. The residue is then diluted with water, heated on the water-bath, animal charcoal added, the solution filtered and the precipitate washed with hot water, the combined filtrates cooled and diluted to a known volume. In a small amount of this solution, an estimation of chlorine is then made, and exactly the necessary amount of sodium hydroxide added to neutralise the hydrochloric acid. This solution is then evaporated to a small volume under diminished pressure, and placed on ice for several days to permit the tyrosine and cystine to crystallise out.

The crystallisation of tyrosine and cystine is collected and washed with ice cold water. It is then dissolved in hot dilute ammonia, cooled and neutralised with acetic acid. The tyrosine is quickly precipitated, though not quantitatively. It is collected, washed with ice water and further treated as described under tyrosine. The filtrate is then made ammoniacal and calcium chloride added to precipitate any phosphoric or oxalic acid that may be present. The filtrate is then mixed with an equal volume of acetone and the mixture acidified with acetic acid, following which the cystine will separate out, to be further treated as described under cystine.

The filtrate after the separation of the tyrosine and cystine is then evaporated to a small volume under diminished pressure, the residue taken up in absolute alcohol and again evaporated to a syrup, care being taken not to continue the process after bubbles form in the syrup. Three or four volumes of absolute alcohol are then added (care must be taken that a large flask is selected) and gaseous hydrogen chlo-

ride passed into the solution until it is saturated. Care must be taken that the gas is absolutely dry. Heat is applied during the process; finally the passage of gas is continued until the cooled alcoholic solution is saturated. In this process the amino-acids have been esterified and all pass into solution.

This alcoholic solution is now concentrated under diminished pressure, at a temperature of not over 40°, to about one-third or one-fourth of its volume and placed on ice for 1 or 2 days, to permit the hydrochloride of the ester of glycocoll to crystallise out; this is much facilitated by inoculation with a crystal of the substance. Care must be taken during these operations to prevent absorption of water by the alcohol. The crystallisation of the salt of the ester of glycocoll is then collected by filtration, washed with cold absolute alcohol and treated further as will be described under glycocoll.

The alcoholic filtrate is now evaporated to dryness at 40° under diminished pressure and absolute alcohol again added as before and the process of esterification repeated by another introduction of gaseous hydrogen chloride. The solution is again treated exactly as before, to secure the separation of any further glycocoll, the solution being more concentrated before being placed on ice. The filtrate may then be submitted to the processes for the separation of the esters, or the process of esterification may be again repeated, and even again, if the attempt is being made to secure as nearly as possible the complete yield.

The final filtrate is evaporated to dryness at 40° and the residue taken up in about 3 volumes of absolute alcohol, filtered and the chlorine content determined in a small fraction. The calculated needed amount of metallic sodium (excess to be carefully avoided) is then added to the amount of absolute alcohol necessary to make a 3 % solution, and the cooled solution of sodium ethoxide added to the solution of esters. A little ether is then added and the mass placed on ice over night. The sodium chloride is precipitated and is to be filtered off.

This solution is then subjected to fractional distillation. It is first distilled at 40° at 10 mm. pressure until all the alcohol-ether has passed over. The esters of glycocoll are not completely removed by crystallisation and d-alanine distils over in this fraction. The distillate is mixed with dilute hydrochloric acid, the separated ether removed and the acid solution which now contains the hydrochlorides of the named amino-acids evaporated to dryness. Polariscopic ex-

amination of the aqueous solution of this residue will fix the presence
of *d*-alanine. If it be present, then the residue is to be esterified just
as before, and the hydrochloride of the ester of glycocoll removed by
crystallisation as before. The filtrate will contain now fairly pure
d-alanine; the ester is hydrolysed by boiling in 10 parts of water and
the *d*-alanine further treated as described under that heading.

A new receiver having been attached, the distillation of the mixture
of esters is continued, the heat being slowly raised to 60°, the pressure
maintained at 10 mm. When nothing further comes over, another
new receiver is attached and the heat raised slowly to 100°. The
distillates are cooled with ice, and the distillation continued at 100°
so long as there is a yield. The distilling flask is now transferred to
an oil-bath, the pressure reduced to less than 1 mm. and the distillation
continued at 100° so long as there is a yield. Finally, with a fourth
receiver, with the pressure maintained below 1 mm., the distillation
is continued with the temperature gradually raised to 180°, in fractions
of about 20° each, until there is no further yield. We now have there-
fore 4 distillation fractions. In I, II and III may be found mixtures
of proline, *d*-alanine, *d*-valine, *l*-leucine (possibly isoleucine) and such
traces of glycocoll as may have escaped previous removal. In frac-
tion IV may be found the esters of serine, phenylalanine, aspartic acid
and such glutamic acid as may have escaped the previous crystallisa-
tion. While some leucine will be in all the first three fractions, most
of it will be in the second and third, with the valine and possibly iso-
leucine. In the first fraction will be found most of the alanine with
the glycocoll. Proline is to be recovered from all three.

The first three fractions are to be treated alike, though separately.
The distillates are suspended in 10 parts of water and boiled under a
reflux condenser to complete saponification, until the alkaline reaction
disappears, this being accomplished in from 6 to 10 hours. The solu-
tion is then evaporated at low temperature and low pressure to a small
volume and allowed to remain over night for a possible crystallisation
of leucine. If such occurs, the crystals should be collected, and added
to those later to be secured. Then the evaporation is continued to dry-
ness. The dried residue is then extracted with boiling absolute alco-
hol, in which the proline is soluble, the others not to any great extent.
These extractions are thrice repeated and the collected alcoholic ex-
tracts set aside to cool. On the following day the extract is filtered,
and the residue extracted repeatedly with boiling absolute alcohol.

After cooling, the final residue is returned to the original residue of each fraction. The final alcoholic extracts of the three fractions are now joined and evaporated to dryness. When taken up again in absolute alcohol, any further insoluble residue must be rejected. This alcoholic extract of proline is then evaporated to dryness and further treated as described under that heading.

The residues of the three fractions are now dissolved in hot water, filtered through animal charcoal, concentrated to small volume and set aside for crystallisation. The first crystallisation will be largely leucine, especially in fractions II and III. The filtrates are further concentrated and set aside for crystallisation; and this is repeated the third time, to the almost complete driving off of the water of solution. The first crystallisations are usually largely leucine, and to these should be added the previously secured yield of leucine. The second and third crystallisations of fraction I will consist largely of alanine and glycocoll, with a little leucine. The second and third crystallisations of fractions II and III will consist largely of valine and leucine, with possibly some isoleucine. There is, however, overlapping in all the crystallisations. d-valine is soluble in methyl alcohol, and may be often secured in this manner, especially in the absence of isoleucine. Glycocoll may be secured by repetition of the esterification and crystallisation of the hydrochloride of the ester. The different fractions of crystals are then to be tested for the several compounds and derivatives that will be described under the different headings.

The distillate contained in fraction IV is taken up in 5 volumes of water and shaken with ether in a separating funnel until the water solution is clear, the ester of phenylalanine passing into the ether. The ether extract is then separated and washed several times with water, the waters being returned to the original aqueous solution. The ether is evaporated to dryness, the residue taken up in dilute hydrochloric acid and again evaporated to dryness, in which process saponification occurs. The hydrochloride may then be secured by crystallisation, and subjected to the tests for the substance to be described under that heading.

The aqueous solution is then saponified by boiling with barium hydroxide, using about twice the amount of the weight of the distillate. The mixture is boiled for 2 hours under a reflux condenser, and then set aside over night. The barium salt of racemic aspartic acid separates out, is collected on a filter and washed with barium hydroxide solution. The salt is then suspended in 25 % sulphuric acid and

boiled, filtered, and the excess of sulphuric acid removed by the careful addition of barium hydroxide and again filtered. From the filtrate aspartic acid may be recovered by crystallisation. This is to be added to the fraction later to be secured and treated further as will be described under that heading.

The filtrate remaining after removal of the barium salt of aspartic acid is freed of barium with sulphuric acid, evaporated to dryness under diminished pressure, the residue dissolved in hot water, filtered through animal charcoal and the solution saturated with gaseous hydrochloric acid. On standing the hydrochloride of glutamic acid crystallises out. This is collected, joined to the fraction that was early obtained, recrystallised from dilute hydrochloric acid and identified as described under that heading.

The filtrate contains the rest of the aspartic acid and serine. It is evaporated to dryness under diminished pressure, and for the better removal of the hydrochloric acid the residue again taken up in water and again evaporated to dryness. This residue is taken up in water, the chlorine removed by the controlled addition of yellow lead oxide with boiling, filtered, the excess of lead removed with hydrogen sulphide and the filtrate concentrated, following which the aspartic acid will separate out. The filtrate contains the serine in the racemic state, and on careful neutralisation and further concentration much of the serine will crystallise out. The serine is then further to be tested by the methods described under that heading.

The methods are not quantitative. By weighing it is possible in the different stages to get an idea of the amounts concerned, but losses occur with every amino-acid and in every stage.

When investigating a material containing preformed amino-acids, the material should be evaporated to dryness, extracted with hot hydrochloric acid, sp. gr. 1.15, filtered and then set aside for a possible crystallisation of glutamic acid. Following this, the material is treated exactly as described. This is the best method for the analysis of urine; the direct precipitation of the amino-acids, as sometimes advised, by β-naphthalene-sulpho-chloride is liable to fail even if the amino-acids be present in abundance.

ISOLATION OF DIAMINO-ACIDS

For the isolation of histidine and the diamino-acids, arginine and lysine, it is better to use a separate lot of the material. The material is extracted in hot acidified water, if one is to search for the preformed

substances. If the material be a protein, it is to be hydrolysed as previously described; sulphuric acid of 25% strength is preferable, and the acid removed by precipitation with barium sulphate, filtered and washed five times with hot water. The material is now to be treated alike in each instance. Sulphuric acid is added to make a concentration of 5%, and a solution of 10% phospho-tungstic acid in 5% sulphuric acid is added so long as a precipitate forms. This precipitates histidine, arginine and lysine; but the amino-acids are not precipitated from dilute solution. The precipitate is washed with dilute sulphuric acid, suspended in water, the metal precipitated by the addition of barium hydroxide until the reaction is alkaline, filtered and the precipitate thoroughly washed, the excess of barium removed from the filtrate by the addition of sulphuric acid to the point of distinct acidity, and again filtered and the precipitate washed. The filtrate is then transferred to a large flask and a boiling hot solution of silver sulphate added, with vigorous shaking, until a drop of the supernatant liquor gives a brownish reaction when dropped into a watch-glass containing a solution of barium hydroxide. The mixture is then cooled and saturated with powdered barium hydroxide, using so much that an excess lies with the reaction-precipitate in the bottom of the flask. This is then collected on a Buchner funnel, the precipitate is then rubbed up in a mortar with purified sand and a saturated solution of barium hydroxide, again collected on the Buchner funnel, thoroughly washed with barium hydroxide and the filtrates and wash waters collected. The filtrate contains the lysine, the precipitate, the arginine and histidine.

The precipitate is suspended in water and thoroughly mixed, sulphuric acid being added to acid reaction. Sulphuretted hydrogen is then passed in to precipitate the silver, and when this is completed the mixture is heated to steaming and filtered hot, the precipitate extracted and washed with hot water repeatedly, until no reaction with phospho-tungstic acid remains, and the combined filtrates concentrated to a known volume. A Kjeldahl estimation of nitrogen is then made. The solution is carefully neutralised with barium hydroxide solution and then a solution of barium nitrate added drop by drop so long as a precipitate forms. This precipitate of barium sulphate is filtered off and washed, and the filtrate concentrated if necessary. It is then acidified with nitric acid, and silver nitrate in solution added until a drop of the supernatant fluid produces a brownish reaction with

barium hydroxide solution. Then the solution is carefully neu-
tralised with barium hydroxide solution, a little suspended barium
carbonate added, the mixture heated to boiling and then stood aside
to cool. Histidine is precipitated as the silver compound; the silver
compound of arginine remains in solution at neutral reaction. The
precipitate is then collected on a filter and washed with a very dilute
baryta water until the wash water is free of nitric acid, and the
filtrates collected. The precipitate is then heated in water and
sulphuric acid added to the point of an acid reaction, the silver
precipitated with hydrogen sulphide, filtered, washed thoroughly
and the collected filtrates concentrated and the histidine content
estimated by a nitrogen determination. The histidine in the solu-
tion is then to be further treated as described under that heading.

The filtrate containing the soluble silver salt of arginine is saturated
with barium hydroxide, which precipitates the arginine compound.
The precipitate is collected on a Buchner funnel, thoroughly extracted
and washed. The precipitate is then suspended in water, acidified
with sulphuric acid and the silver precipitated with hydrogen sulphide,
the precipitate removed by hot filtration, washed, the collected filtrates
concentrated, the sulphuric acid removed by barium hydroxide, the
excess of barium removed by carbon dioxide and the filtrate concen-
trated and submitted to a Kjeldahl estimation for nitrogen, from which
the amount of arginine is calculated. The solution of arginine is then
to be treated as described under that heading.

The filtrate containing the lysine is acidified with sulphuric acid,
freed from silver by hydrogen sulphide and filtered hot, and the col-
lected filtrates concentrated. Sulphuric acid is then added to make the
concentration about 5 %, and the lysine precipitated by the addition of
phospho-tungstic acid. After 24 hours the precipitate is collected on a
filter, washed with dilute sulphuric acid and the precipitate suspended
in hot water, barium hydroxide added until the reaction is alkaline, the
insoluble salt of tungsten removed by filtration and washed, and the
collected filtrates freed of barium by carbon dioxide, and concentrated
almost to dryness. The residue is taken up in hot water, filtered, and
a nitrogen estimation of the filtrate made, from which the lysine may
be calculated. The solution of lysine is then to be further treated as
described under lysine.

The group reactions of the amino-acids, by means of which we
obtain derivatives upon which we rely for the identification of the

different amino-acids, are carried out as follows. The different amino-acids should be isolated as far as possible, as previously described, since all these derivatives form their characteristic compounds best when pure. The amino-acid solution is made alkaline with sodium hydroxide to about normal alkalinity. Then the solution is placed in a separating funnel, and a goodly amount of a 10% solution of pure β-naphthalene-sulphochloride in ether added and the funnel shaken in a machine. Every hour the reaction of the aqueous solution is tested, and alkali added if necessary to maintain decided alkalinity. After shaking for 10 hours, the aqueous solution which contains the derivative is separated and filtered. It is then acidified and extracted thoroughly with ether, which takes up the derivative. The ethereal extract is then washed with water to remove the acid reaction, and the ether evaporated to dryness. The residue is taken up in hot dilute ammonia, filtered through animal charcoal, the filtrate acidified and again extracted with ether, the ethereal extract washed with cold water and evaporated to dryness. This residue is taken up in hot dilute ammonia, acidified with hydrochloric acid and the compound permitted to crystallise out. Fractional crystallisation may be necessary and subsequent purification.

To the amino-acid dissolved in normal alkali, an amount of phenyl-iso-cyanate equal to that of the amino-acid is added in small portions, the mixture kept cool and vigorously shaken for an hour. Animal charcoal is then added, and the mixture filtered, following which the filtrate is acidified and set on ice. The next day the precipitate is collected, taken up in 4% hydrochloric acid, and concentrated on the water-bath, later to be set aside for crystallisation. With some amino-acids the phenyl-iso-cyanate derivative crystallises well; with others the corresponding hydantoin is to be preferred.
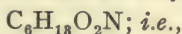
The derivatives of α-naphthyl-iso-cyanate are obtained in the same way, being soluble in alkali and insoluble in acid solution. They may be recrystallised well from hot alcohol.

To form the derivatives with benzoyl-chloride, the solution of amino-acid is made alkaline with sodium carbonate and benzoyl-chloride added, with strong shaking, so long as it is taken up. After a few hours the mixture is filtered, acidified with hydrochloric acid and set aside in a cool place for crystallisation. The precipitate is collected, washed in cold acidified water, dried and the benzoic acid extracted with petroleum ether. The remaining salt may then be

redissolved in alkaline water, and recrystallised from the acidified medium.

To form the carbamino-acid derivative, the solution of the amino-acid is saturated with carbon dioxide, chilled and lime water added, following which carbon dioxide is continuously added until saturated. Then lime water and crystallised calcium carbonate are added and the mixture vigorously shaken. After filtration, chilled alcohol is added to the filtrate until opacity appears. On standing the calcium salt of the carbamino-acid crystallises out.

Leucine (α-Amino-iso-butylacetic Acid).

$$C_6H_{13}O_2N; \ i.e.,$$

$$\underset{\substack{\displaystyle | \\ \displaystyle CH_2 \\ \displaystyle | \\ \displaystyle CH.NH_2 \\ \displaystyle | \\ \displaystyle COOH}}{\overset{\displaystyle CH_3 \ CH_3}{\underset{\displaystyle CH}{\diagdown \diagup}}}$$
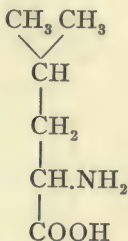
Leucine was originally discovered by Proust (1819) in putrefied cheese. It was found by Liebig in diseased, but not in healthy, calf's liver. It has been found in the brain, pancreas, thyroid and thymoid glands, etc.; and in the liver and urine in cases of small-pox, typhus fever, leucæmia, affections of the spinal cord, sepsis, acute yellow atrophy, and poisoning by phosphorus. Leucine is a characteristic product of the putrefaction of gelatin and proteins, and is produced, together with the other amino-acids, aspartic and glutamic acids, by the action of boiling dilute acids or fused potassium hydroxide on these substances.

In the vegetable kingdom, leucine has been found in young pumpkins, beetroot and beetroot molasses, the juice of vetches germinated in the dark; in *Agaricus muscarius*, etc., etc.

Leucine has been prepared synthetically by the reaction of α-bromohexoic acid with ammonia.

Leucine is conveniently prepared by boiling 2 parts of horn-shavings with 5 parts of sulphuric acid and 13 of water for 24 hours, under a reflux condenser. The product is then treated with excess of lime, boiled well, filtered, and the filtrate evaporated to about one-half.

It is then faintly acidified with oxalic acid, the calcium oxalate filtered off,[1] and the filtrate concentrated till a crystalline film forms on the surface. On cooling, crystalline laminæ are deposited, consisting of a mixture of leucine and tyrosine (Fig. 7), and a further crop can be obtained by concentrating the mother-liquor. The crystals are redissolved in such a quantity of boiling water that only tyrosine is deposited on cooling. The mother-liquor is treated with precipitated lead hydroxide, which removes colouring matter and a little tyrosine, and the filtrate freed from lead by hydrogen sulphide and evaporated till a film forms on the surface. The crystals of leucine which deposit on cooling may be further purified from tyrosine by treatment with boiling alcohol of 70% (sp. gr., 0.872), which leaves the tyrosine undissolved. To get rid of traces of a sulphuretted impurity, the leucine may be dissolved in dilute alkali hydroxide, a solution of lead oxide in caustic alkali added, and the liquid boiled for half an hour. The liquid filtered from the lead sulphide is exactly neutralised by sulphuric acid, evaporated to complete dryness, and the residue exhausted with boiling

Fig. 7.—Tyrosine and Leucine as obtained together in the process of preparation.

alcohol of 0.830 sp. gr., which on cooling deposits the leucine in a state of absolute purity. The crystals melt with decomposition at 293°.

Leucine separates from alcohol in thin nacreous scales resembling cholesterol. The crystals have a sp. gr. of 1.293, but are wetted with difficulty by water, and often float on the surface. When not perfectly pure, leucine often separates in concentric nodules closely resembling fatty cells (Fig. 8), but which under the microscope appear as concentrically grouped, highly refracting needles.

When cautiously heated (in a tube open at both ends), leucine sublimes unchanged in light white flocks, which under the microscope are seen to consist of delicate scales grouped together in the form of

[1] At this stage Waage gradually adds recently precipitated cupric hydroxide (avoiding excess), and boils. On cooling, a copper compound of leucine separates out in light blue scales. This is filtered off, decomposed by sulphuretted hydrogen, and the liberated leucine purified by crystallisation from dilute alcohol.

rosettes. When heated to 170°, leucine melts to a brown viscous liquid, and at a slightly higher temperature decomposes into amylamine and carbon dioxide: $C_6H_{13}O_2N = C_5H_{13}N + CO_2$.

Leucine is sparingly soluble in cold water 1:40, but more readily in boiling water. It requires 660 parts of cold 50% alcohol and 1040 parts of cold alcohol of 96% for solution, but dissolves in 800 parts of boiling 98% alcohol, and more readily in weaker spirit. Its solubility in water and in alcohol is increased by acetic acid or an acetate of alkali-metal. Leucine is insoluble in ether. It dissolves readily in acids and alkalies.
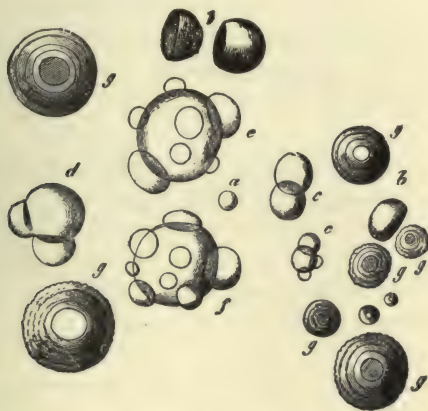
Fig. 8.—Spheroidal crystalline masses of leucine. *a*, a very minute simple spherule; *b*, hemispheroidal masses; *c c*, aggregates of small globules; *d*, a large globule supporting two halves; *e f*, a large spheroid of leucine richly studded with minute segments; *g g g g*, laminated globules of leucine, some with smooth, some with rough surface, and of very various sizes.

Leucine is optically active; the *l*-leucine naturally obtained rotates the plane of polarised light to the left in aqueous solution ($[\alpha]_D = -10.4°$), but to the right in hydrochloric acid solution, $[\alpha]_D = +15.6°$.

A variety of leucine has been obtained by Schulze and Likiernik from the vegetable protein conglutin. When heated with barium hydroxide to 160° it loses its optical activity, and forms an inactive modification which has been proved to be identical with the α-amino-isobutyl-acetic acid prepared synthetically from iso-valeraldehyde. Both compounds have the same solubility in water, yield active

leucine under the action of *Penicillium glaucum*, and are converted into the same hydroxycaproic acid on treatment with nitrous acid.[1]

When fused with alkali hydroxide, leucine yields normal valeric acid, $C_5H_{10}O_2$, ammonia, hydrogen, and carbon dioxide. When heated with fuming hydriodic acid to 140° it yields caproic acid and ammonia, $C_6H_{13}ON_2 + H_2 = C_6H_{12}O_2 + NH_3$. Nitrous acid decomposes leucine into nitrogen and hydroxycaproic or leucic acid, $C_5H_{10}(OH).CO.OH$.

When leucine is heated with nitric acid on platinum foil, a colourless residue is left, which becomes yellow on addition of a drop of sodium hydroxide; and on careful evaporation this forms an oily drop which does not wet the platinum. This reaction was observed by Scherer (*Jahresber.*, 1857, 541), and is said to be characteristic.

When heated for some time with excess of nitric acid, leucine is entirely converted into gaseous products; but as long as the decomposition is incomplete the remaining portion consists of nitrate of leucine.

Heated with strong sulphuric acid, leucine is decomposed, the whole of the nitrogen being converted into ammonia. By potassium permanganate it is converted into valeric acid, oxalic acid, and ammonia. The two preceding reactions have a practical interest in connection with Kjeldahl's process of determining nitrogen.

An aqueous solution of leucine is coloured a deep red by ferric chloride.

Leucine is amphoteric in reaction, but in its capacity of an amino-acid forms crystallisable compounds both with acids and bases, and also unites with neutral salts in a manner similar to glycocoll (page 211).

B,HCl forms crystalline scales, very soluble in water. B_2,H_2PtCl_6 forms yellow crystalline grains, soluble in water, but insoluble in alcohol. B,HNO_3 forms colourless needles, very readily soluble in water.
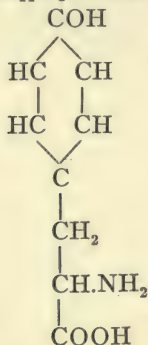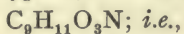
Copper amino-caproate, $Cu(C_6H_{12}O_2N)_2$, is obtained by adding

---

[1] According to Schulze and Bosshard (*Zeit. physiol. Chem.*, 10, 134), the leucine obtained from the vegetable protein conglutin is optically active when hydrochloric acid has been employed as the decomposing agent, but inactive if barium hydroxide solution at a temperature of 150–160° has been used. When leucine obtained by the former method is heated with barium hydroxide solution under pressure at 150–160° for 3 days, it becomes optically inactive, and its solubility in water diminishes. Heating leucine with water alone under the same conditions produced no change. When the mould *Penicillium glaucum* was grown in a solution of leucine for five or six weeks, the optical activity was exactly reversed, $[a]_D$ changing from $+17.3°$ to $-17.3°$ (See also E. Schulze, *Ber.*, 1893, 26, 56).

Schulze and Likiernik (*Zeit. physiol. Chem.*, 17, 513) find that the leucine obtained from fibrin, elastic tissue, and other sources has the same properties as that derived from conglutin. All the leucines known are either *d-*, *l-* or *dl-* leucines; there are no other isomers

recently precipitated cupric hydroxide to a strong aqueous solution of leucine, and boiling the liquid. A bluish solution results, which on cooling deposits light blue scales, which require 3,045 parts of cold or 1,460 parts of boiling water for solution. This reaction may be employed for the isolation of leucine, but the solubility of the copper compound is materially increased by the presence of certain organic matters. With excess of copper oxide, leucine forms an insoluble compound. $Pb(C_6H_{12}O_2N)_2 + H_2O$ is deposited in mirror-like plates or nacreous scales, on cautiously adding ammonia to a boiling solution of leucine to which lead acetate has been added. In the isolation of leucine as described under the ester method, several fractions of crystals of leucine are secured, partly mixed with valine and possibly with alanine and even glycocoll. Fractional crystallisation must be relied upon most largely to secure the pure leucine. The hydrochloride of the ethyl ester melts at 134°. The ester forms also a picrate that melts at 128°. The most characteristic derivative of leucine is the α-naphthyl-iso-cyanate, long thin needles, melting at 263°. β-naphthalene-sulpho-leucine crystallises in fine prisms that melt at 67° to an oily drop. The phenyl-iso-cyanate is to be recrystallised from warm alcohol by the addition of warm water, and forms flat leaves and prisms that melt at 165° with formation of gas.

Tyrosine (hydroxyphenyl-α-aminopropionic acid).

$C_9H_{11}O_3N$; *i.e.*,

$$
\begin{array}{c}
\text{COH} \\
\diagup \quad \diagdown \\
\text{HC} \quad \text{CH} \\
|\qquad| \\
\text{HC} \quad \text{CH} \\
\diagdown \quad \diagup \\
\text{C} \\
| \\
\text{CH}_2 \\
| \\
\text{CH.NH}_2 \\
| \\
\text{COOH}
\end{array}
$$

Tyrosine occurs ready-formed, and almost always accompanied by leucine, in both the animal and vegetable organisms.[1] It is likewise

[1] Tyrosine is said to occur with leucine in spiders, caterpillars, and moths; but not in butterflies, which contain leucine only. It occurs in the cochineal insect, and in the pancreas and other organs of mammals.

produced, together with leucine, by the putrefactive decomposition of proteins, or by their treatment with alkalies or acids, and has also been obtained synthetically.

The proportions of tyrosine and leucine yielded on boiling proteins with diluted sulphuric acid are given on page 270.

A method of preparing tyrosine is described on page 227. Another plan is to gradually add dry casein, fibrin, or albumin (free from fat) to an equal weight of fused or highly concentrated solution of potassium hydroxide contained in a capacious iron vessel. The heating, which isaccompanied by an evolution of ammonia and most disagreeable odour, is continued until evolution of hydrogen commences, and the fused mass changes in colour from brown to yellow. The product is then poured out, dissolved in hot water, and the solution slightly acidified with acetic acid. On cooling, an abundant crop of crystals of tyrosine separates in concentric groups of needles, which may be purified by re-solution in hot water containing potassium carbonate and precipitation with acetic acid.

For the further purification of tyrosine, it should be dissolved in a known quantity of hydrochloric acid, the solution treated with animal charcoal, and filtered. The hot filtrate is treated with sodium or potassium acetate in amount equivalent to the hydrochloric acid used, when the greater part of the tyrosine separates on cooling. A product much freer from inorganic matter is obtainable in this way than when crystallisation is effected in a neutral solution. The tyrosine should be recrystallised from hot water containing acetic acid. For the removal of obstinately adhering cystine, Städeler adds to the warm aqueous solution a small quantity of basic lead acetate, treats the filtered liquid with hydrogen sulphide, and recovers the tyrosine by concentrating the filtrate to the crystallising point.

Tyrosine is deposited from its hot aqueous solution in stellate groups of long, slender, silky needles, which on drying readily become felted together to a snow-white mass. From ammoniacal solutions it is deposited in larger needles, also having a silky lustre (Fig. 9).

Tyrosine is tasteless, odourless, and infusible. When heated, it evolves an odour of burnt bones. Heated cautiously to 270°, it gives off carbon dioxide, and yields a white sublimate of hydroxyethyl-aniline, $C_2H_4(OH).NH.C_6H_5$.

Tyrosine is soluble in 2,400 parts of cold or 150 parts of boiling water. In hot ammonia and in acetic acid it dissolves unchanged,

and is deposited on cooling. It requires 13,500 parts of cold rectified spirit for solution, is not much more soluble on boiling, and is quite insoluble in absolute alcohol and in ether. Its solubility in alcohol is greatly increased by the presence of amorphous extractive matters. Tyrosine may be separated from leucine by the fact of the solubility of the latter in glacial acetic acid mixed with one volume of alcohol. Tyrosine is fermented by certain enzymes called tyrosinases, with the production of blackish colouring matters. This reaction, which is technically best effected with laccase, is probably a normal reaction in the adrenal body.

FIG. 9.—Tyrosine. *a*, single crystals; *b b*, smaller and larger groups.

Natural tyrosine is lævorotatory. The value of $[a]_D$ in solution in fuming hydrochloric acid is $-16°$.

When fused with potassium hydroxide, tyrosine yields ammonia, acetic acid, and para-hydroxybenzoic acid, $C_6H_4(OH).COOH$.

When treated with strong nitric acid, tyrosine is converted into nitrotyrosine nitrate, from the solution of which ammonia precipitates free nitrotyrosine, $C_9H_{10}(NO_2)NO_3$, which crystallises from hot water in light yellow, very sparingly soluble needles, having a slightly bitter but not acid taste, and dissolving in alkali hydroxide with deep red colour. Dinitrotyrosine, $C_9H_9(NO_2)_2NO_3$, is obtained by evaporating tyrosine with excess of nitric acid. It is a well-defined

dibasic acid, forming golden-yellow plates, sparingly soluble in water, but readily in alcohol, having an acid but not bitter taste. The salts deflagrate on heating.

If Millon's reagent be added to a boiling aqueous solution of tyrosine, the liquid acquires a pink or rose-red colour, and red flakes are gradually precipitated.

When tyrosine is gently warmed with strong sulphuric acid, it dissolves with transient red colouration to form tyrosine-sulphonic acid, $C_9H_{10}(SO_3H)N_3O + 2H_2O$. On diluting the solution after a time with water, boiling with barium carbonate or chalk, and gradually adding neutral ferric chloride to the neutral filtrate, a fine dark violet colouration is produced. This reaction, which is due to Piria, affords a very delicate test for tyrosine. Unfortunately, leucine somewhat interferes with it. Denigès' test for tyrosine is often advantageous. The crystals to be tested are added to the reagent, consisting of formaldehyde 1, water 55 and sulphuric acid 55. A green colour develops, characteristic and permanent.

The crude tyrosine obtained in the general method previously described is purified by repeated crystallisation. It may be necessary to remove impurities by precipitation with phospho-tungstic acid. The cystine can usually be removed by fractional crystallisation; the tyrosine is much the least soluble.

The hydrochloride of the ethyl ester forms long prisms that melt at 109°. The α-naphthyl-isocyanate forms small centrally grouped needles m. p. at 205°. The combination with β-naphthalene-sulpho-chloride, which does not respond to Millon's reaction, forms free needles that melt to an oil at 120°.

Asparagine. Aminosuccinamic Acid.

$$C_4H_8O_3N_2; \text{ i.e.,}$$
$$CO.NH_2$$
$$|$$
$$CH_2$$
$$|$$
$$CH.NH_2$$
$$|$$
$$COOH$$
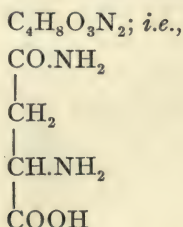
Asparagine was discovered in 1805 in the juice of asparagus. It exists ready-formed in many other plants, including marsh-mallow, comfrey, chestnuts, potatoes, the leaves of the deadly night-shade,

liquorice-root, dahlia-tubers, and is present in comparatively large quantity in the roots of *Robinia pseudacacia*. Asparagine is also found in the milky juice of the lettuce, and in the young shoots of vetches, beans, peas, and other leguminous plants, though the *seeds* of these contain no trace of asparagine. The quantity of asparagine diminishes with the progress of the growth of the plant, and disappears entirely when the seeds are formed. Boussingault found asparagine to be constantly present in plants grown in the dark.

Asparagine may be prepared by dialysing the juice of asparagus, marsh-mallow, or *Scorzonera Hispanica*, concentrating the dialysate to a syrup, and allowing it to stand for some days, when the asparagine will separate in crystals. From liquorice, asparagine may be prepared by exhausting the root with water, boiling to coagulate albumin, treating the filtrate with acetic acid to precipitate glycorrhizic acid, and adding lead acetate to the filtered liquid, to throw down phosphates, malates, colouring matter, etc. The filtrate, when evaporated to a small bulk, deposits crystals of asparagine after standing for some days. Asparagine may also be isolated by treating the filtrate from the lead precipitate with mercuric nitrate, and decomposing the resultant compound with hydrogen sulphide. This plan is especially useful in the presence of soluble carbohydrates, which prevent the crystallisation of the asparagine.

Asparagine forms hard, transparent, rhombic prisms, which have a sp. gr. of 1.519. The crystals belong to the orthorhombic system, and exhibit left-handed hemihedry. They contain one molecule of water which is lost at 100°. The crystals grate between the teeth, and have a slightly cooling, sickly taste.

Asparagine is moderately soluble in cold water (1:82 at 10°; 1:47 at 20°), but much more readily on boiling (1:1.9).[1] It dissolves freely in acid and alkaline liquids. It is insoluble in cold absolute alcohol, and almost insoluble on boiling, and is not dissolved by ether, or by fixed or volatile oils.

Asparagine is optically active, but the extent and direction of the rotation depend on the solvent. Thus a solution of asparagine in water has a specific rotation of about −6°; but by addition of alkalies the activity is increased, in ammoniacal solution the rotation being −11°. In hydrochloric acid solution, (1 mol. HCl) on the other hand,

[1] The solubility of asparagine in cold water is given very variously due to impurities, the statements ranging from 1 in 12 to 1 in 300.

asparagine exhibits a dextro-rotation of about $+26°$. Addition of a small quantity of acetic acid to the aqueous solution of asparagine decreases the lævorotation, and on further addition of acid the liquid becomes dextrorotatory. (For data, see Landolt, *Optische Drehungs vermögen*).

The aqueous solution of asparagine has a faintly alkaline reaction to litmus. When quite pure it can be kept without change, but in presence of protein matter and bacteria the solution soon ferments, the asparagine being completely converted into ammonium succinate:

$$C_4H_4O_3(NH_2)_2 + H_2O + H_2 = C_4H_4O_4(NH_4)_2.$$
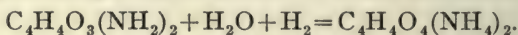
A similar change results when asparagine is taken internally, the urine after asparagus has been eaten acquiring a peculiar odour and containing ammonium succinate.

Asparagine exhibits both an acid and a basic function. The hydrochloride, $C_4H_8O_3N_2,HCl$, forms large, readily soluble crystals. $Cu(C_4H_7O_3N_2)_2$, is obtained by treating a solution of asparagine with cupric hydroxide or cupric acetate.

Asparagine reduces Fehling's solution on boiling, which reaction distinguishes it from glutamine.

When asparagine is dissolved in cold nitric acid (free from nitrous acid) it is converted into aspartic acid and ammonium nitrate; but if nitrous acid be present, or if nitric oxide or nitrous fumes be passed into the solution, the aspartic acid is converted into malic acid, with evolution of nitrogen, the reaction being, according to Sachsse and Kormann:

$$C_4H_8O_3N_2 + 2HNO_2 = C_4H_6O_5 + 2H_2O + 2N_2.$$
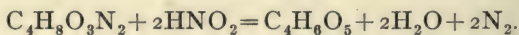
The most characteristic reaction of asparagine is its conversion into aspartic acid and ammonia by treatment with alkalies or mineral acids. The change readily occurs when asparagine is boiled with water and lime, baryta, or litharge; or with dilute hydrochloric or sulphuric acid:

$$C_4H_8O_3N_2 + H_2O = C_4H_7O_4N + NH_3.$$
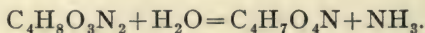
The reaction might possibly be made quantitative, but B. Schulze has shown that there is a tendency to further decomposition if the action be too prolonged. Boiled with water alone at the atmospheric pressure for 12 hours, only 2 % of the asparagine was converted into ammonium aspartate. Under higher pressure the conversion was much greater. Milk of lime had no action in the cold after

24 hours, but on boiling with lime or barium hydroxide the action was much more rapid. When a large excess of baryta was used, the conversion was complete in 1 hour, but on continuing the treatment some hours more a further elimination of ammonia occurred, with formation of malic acid. Boiling with water containing one-tenth of its volume of strong hydrochloric acid effected complete conversion in one hour, action on the aspartic acid occurring if the treatment were further prolonged. Schulze also obtained good results by treating 2 grm. of asparagine with 5 c.c. (= 8.79 grm.) of pure sulphuric acid and 100 c.c. of water, and boiling under a reflux condenser for 2 hours. The cooled liquid was nearly neutralised with soda and distilled with magnesia. The ammonia found in the distillate was fairly in accordance with theory.

A method of determining asparagine in plant-products has been based on this reaction by R. Sachsse (*Jour. prak. Chem.*, [ii], **6**, 118); but for this purpose it is necessary previously to get rid of various co-occurring matters. Sachsse boils 10 grm. of the powdered substance for 15 minutes with 200 c.c. of a mixture of equal volumes of alcohol and water, under a reflux condenser. 5 c.c. of a cold saturated solution of mercuric chloride[1] in alcohol is diluted with an equal measure of water and added to the decoction while still hot, and the liquid filtered, the residue being washed first with proof-spirit and then with cold water.[2] The filtrate is evaporated to dryness, the residue taken up in the minimum quantity of hot water (not more than 50 c.c.), and hydrogen sulphide passed through the filtered liquid, without filtering. The filtrate from the precipitated mercuric sulphide is then brought to a volume of 100 c.c., 10 c.c. of hydrochloric acid added, and the liquid boiled under a reflux condenser for 1 hour. The liquid is neutralised by soda and distilled with magnesia, the ammonia in the distillate being determined by titration with standard acid. 17 parts of ammonia or 14 of nitrogen resulting from the treatment with hydrochloric acid correspond to 150 parts of crystallised asparagine originally present.

Estimations of asparagine by Sachsse's method in young lupines were found by Schulze and Barbieri to agree very nearly with the quantity obtained by crystallisation. Amino-compounds generally are, in

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[1] Schulze (*Ber.*, **15**, 2255) employs mercuric nitrate in place of mercuric chloride for the precipitation of asparagine, and for its separation from carbohydrates.
[2] The washing may be avoided by making up the liquid to 500 c.c., passing it through a dry filter, and evaporating 400 c.c. of the filtrate (= 8 grm. of material).

their opinion, estimated with more certainty by the method of Sachsse and Kormann (*Zeits. anal. Chem.*, **14,** 380), in which the asparagine, etc., is first decomposed by boiling with dilute acid, and the resultant amino-acids, after removal of ammonium salts, treated with potassium nitrite and dilute sulphuric acid, the evolved nitrogen being measured. By this treatment all known amino-compounds are decomposed. Proteins and peptones must be removed before the amino-compounds can be determined. The following are the proportions of nitrogen in different forms found by Schulze and Barbieri in certain plant-products. The peptone-nitrogen, B, is that thrown down by phospho-tungstic acid in the filtrate from the albuminoids. D is the difference between the total nitrogen and that existing in the A, B, and C forms. These fractions overlap.

Substance	Protein nitrogen A	Peptone nitrogen B	Amino nitrogen C	Unknown forms D	Total nitrogen E
	%	%	%	%	%
Lupine seeds	8.17	0.24	....	....	8.63
Soja beans	6.32	0.13	....	....	6.73
11–12 days old sprouts of lupine.	3.40	1.60	....	....	10.64
12 days old sprouts of lupine ...	2.33	2.17	5.59	0.42	10.51
15 days old sprouts of Soja beans	3.86	0.56	2.47	0.53	7.42
Birch leaves.................	3.11	0.15	0.66	0.40	4.32
Young grass.................	1.55	0.21	0.22	0.20	2.22

C. Böhmer (*Landw. Versuchs. Stat.*, **28,** 247; abst. *J. Chem. Soc.*, 1883, 237) has given the following data respecting the mode of occurrence of nitrogen in various vegetables, which were cut when fit for use. The figures are percentages, and refer to the moisture-free substances. The water ranged from 4.3 in the truffles to 96% in the asparagus.

Vegetable	N as protein	N as amino-acid amide	N as amino-acid	N as ammonia	Total nitrogen
Spinach	3.51	0.123	0.068	0.021	4.56
Peas	3.56	0.052	0.361	0.020	4.69
Beans	4.39	0.027	0.059	0.013	5.57
Asparagus	3.33	....	....	?	4.13
Lettuce............	2.97	0.155	0.154	0.024	4.85
Carrot.............	1.57	0.013	0.142	0.006	1.91
Turnip-cabbage.....	2.05	0.151	0.231	0.018	4.64
Cauliflower.........	2.60	0.104	0.566	0.017	5.11
French beans.......	2.67	0.061	0.442	0.010	4.32
Mushrooms	3.34	0.092	0.416	0.011	4.68
Truffles............	3.63	0.072	0.202	0.008	4.50

In the foregoing analyses, the ammonia was determined by milk of lime, in the manner recommended by Schloesing and modified by Schulze and Emmerling, and weighed as platinichloride. To determine the amino-acids and acid amides, the proteins were precipitated by Stützer's method with cupric hydroxide, and the filtrate concentrated and divided into three parts. One of these was treated at once with hypobromite; the second was boiled for 2 hours with hydrochloric acid, neutralised, and treated with hypobromite. The difference between the volumes of nitrogen evolved in the first and second experiments represents the nitrogen evolved from ammonia produced by the hydrolysis of the asparagine and glutamine. The third portion was boiled first with hydrochloric acid, and next with alkali hydroxide to volatilise the ammonia. It was then treated with nitrous acid for the estimation of the aspartic and other amino-acids, the nitric oxide, etc., evolved with the nitrogen being absorbed by a strong solution of permanganate.

For the isolation of asparagine and glutamine from vegetable juices and extracts, E. Schulze (*Zeits. Anal. Chem.*, **22**, 325) precipitates the liquid with basic lead acetate. The filtered solution is then treated with a neutral solution of mercuric nitrate (best made by adding sodium hydroxide to an acid solution until it no longer reddens methyl-orange). The white flocculent precipitate is filtered off, washed with cold water, and decomposed by hydrogen sulphide. The filtered liquid, boiled to free it from hydrogen sulphide, will, if asparagine or glutamine be present, evolve ammonia when boiled with alkali hydroxide and will dissolve cupric hydroxide to a deep blue solution. Allantoin is also precipitated by mercuric nitrate, but does not dissolve cupric hydroxide, and is precipitated on adding silver nitrate and ammonia. Xanthine, if present, will also be thrown down by the mercury. For the actual isolation of the amides, the filtrate from the mercuric sulphide precipitate should be neutralised with ammonia and evaporated to a small bulk, when asparagine and glutamine will be deposited in crystals on cooling. Or the original plant-juice, after boiling and filtering from coagulated protein, may be acidified with sulphuric acid, and the peptones and ammonia precipitated by phosphotungstic acid. After standing 2 hours, the precipitate is filtered off, and the asparagine and glutamine estimated in the filtrate by boiling with dilute hydrochloric acid, and the ammonia estimated formed by distillation with magnesia.

Other amino-compounds occur in plants, and are more or less liable to be estimated as asparagine unless special means are taken to separate them. E. Schulze, to whom the existing knowledge on the subject is largely due, finds the exact nature of the amino-compounds to vary with the plant under examination, and its age and conditions of life. In the *Caryophyllaceæ* and *Filices* asparagine is entirely replaced by its homologue glutamine.[1]

Aspartic acid,

$$\begin{array}{c} COOH \\ | \\ CH_2 \\ | \\ CH.NH_2 \\ | \\ COOH \end{array}$$
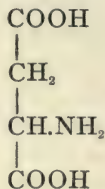
has the constitution of an aminosuccinic acid. It occurs in beetroot molasses, doubtless as a product of the decomposition of asparagine, in spent winelees or vinasse, and in other vegetable juices.[2] It is also formed by boiling albumin or casein with dilute sulphuric acid, by the action of stannous chloride on horn, by treating proteins with bromine, etc. It is secreted preformed by certain sea-snails.

Aspartic acid is best prepared by the hydrolysis of asparagine. H. Schiff (*Ber.*, **17**, 2929) recommends that 100 grm. of asparagine should be boiled for 2 or 3 hours under a reflux condenser with 408 c.c. of hydrochloric acid, containing 48.65 grm. of hydrogen chloride; that is, sufficient for the reaction:

$$C_4H_8O_3N_2,H_2O + 2HCl = C_4H_7O_4N,HCl + NH_4Cl.$$
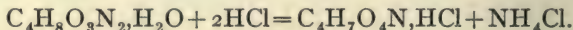
To the cooled solution is added about 200 c.c. of ammonia, containing an amount of real ammonia sufficient to neutralise just one-half the acid previously employed. (The other half has been neutralised by the

[1] In *Lupinus luteus*, Schulze found asparagine, phenylalanine, aminovaleric acid, arginine, choline, and xanthine-like substances; in *Cucurbita pepo*, glutamine, asparagine, leucine, tyrosine, arginine, choline, vernine, and xanthine-like substances; in *Vicia sativa*, asparagine, phenylalanine, leucine, aminovaleric acid, guanidine, choline, and betaïne. This does not indicate that in plant-metabolism the protein molecule breaks down in different ways, it being contended that the disintegrative metabolism of protein is qualitatively the same, but varies quantitatively. This view is supported by experiments on plants of the same kind, but of different ages. Schulze suggests that in some plants certain varieties of nitrogenous crystalline compounds are used more in nourishing the tissues, while in other plants other compounds are more advantageous, and so are used up first.

[2] For the isolation of aspartic acid, the boiling liquid containing it should be treated with carbonate of barium or lead, and alcohol added as long as further precipitation occurs. The precipitate is treated with water, and the barium or lead aspartate reprecipitated by addition of alcohol. The precipitate is again dissolved in water, the barium or lead precipitated with dilute sulphuric acid, and the filtered liquid evaporated to the crystallising point. The crystals are purified by treatment with 60% spirit, and the residue boiled with water, when pure aspartic acid crystallises out on cooling.

ammonia formed in the hydrolysis.)   On cooling the liquid and allow-
ing to it stand, aspartic acid separates in colourless crystals.

Aspartic acid forms small rectangular plates, having a sp. gr.
of 1.66.   It dissolves in about 360 parts of cold water, or in 19
of boiling water, and hence is much less soluble than asparagine.   In
alcohol it is nearly insoluble.   The solutions of aspartic acid in
alkalies are lævorotatory, while those in hydrochloric acid exhibit
dextro-rotation ($[\alpha]_D = +30°$ to $+34°$, according to the proportion of
hydrochloric acid present, see Landolt, *Op. Cit.*).[1]

Aspartic acid forms a series of crystallisable salts with bases.   The
cupric salt, $CuC_4H_5NO_4$, $4\ 1/2\ H_2O$, forms blue needles, soluble
in hot water, but very sparingly soluble in cold water, (1:2800).
This fact may be employed for the detection and isolation of as-
partic acid, solutions of which may be precipitated by cupric
acetate (see Lewinsky, *Chem. Centralb.*, 1894, **1**, 53).

Aspartic acid reduces Fehling's solution.

Aspartic acid is not decomposed by alkaline hypobromite solution,
but by treatment with nitrous acid it is converted into malic acid
with evolution of nitrogen:

$$C_4H_7O_4N + HNO_2 = C_4H_6O_5 + H_2O + N_2.$$
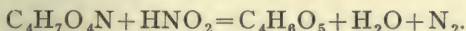
This reaction is employed by Sachsse and Kormann for the estima-
tion of aspartic acid, and, indirectly, of asparagine.   In practice,
sodium nitrite and dilute sulphuric acid are substituted for nitrous
acid.

F. Meunier (*Ann. Agronomiques*, **6**, 275; *Chem. Soc.*, **40**,
761) finds that the estimation of asparagine by measurement of
the nitrogen evolved by the action of nitrous acid is inaccurate.   He
has devised the following process, which depends upon the production
of potassium aspartate and ammonia, when asparagine is treated
with potassium hydroxide.   The crushed, dried, and weighed sub-
stance is placed in a little bag with meshes small enough to retain
the starch.   This is placed in a porcelain dish, exhausted with boiling
water, the filtered solution is heated with subacetate of lead to pre-
cipitate proteins and leucine, and the excess of lead is removed
from the filtrate by sodium hydrogen carbonate.   The filtrate from

[1] Several optically inactive modifications of aspartic acid have been produced by syn-
thetical means.   By heating an aqueous solution of the hydrochloride of the active acid to
170–180° for some hours, an inactive acid is formed which is identical with that obtained
from the ammonium salts of malic, maleic, or fumaric acid.   A lævorotatory aspartic acid
has been prepared from dextro-asparagine by treatment with hydrochloric acid.   Its
properties are the same as those of ordinary aspartic acid, with which it combines to form
an inactive acid (see *Comp. rend.*, **106**, 1734).

the lead carbonate is distilled with alkali hydroxide, and the ammonia in the distillate titrated with standard acid. Since ammonium salts are present, they must be separately estimated and subtracted.

The crude crystals collected as previously described are to be purified by passing through the cupric salt. The benzoyl compound crystallises in shining plates that lose the water of crystallisation at 110°, and melt at 165°.

Glutamine.   Aminoglutamic Acid.

$$C_5H_{10}O_3N_2; \text{ } i.e.,$$

$$
\begin{array}{l}
CO.NH_2 \\
\mid \\
CH_2 \\
\mid \\
CH_2 \\
\mid \\
CH.NH_2 \\
\mid \\
COOH
\end{array}
$$
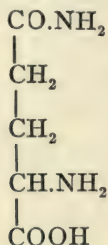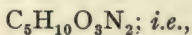
Glutamine is the higher homologue of asparagine, and coexists with it in beetroot, pumpkins, and the shoots of vetch. In the families *Caryophyllaceæ* and *Filices* glutamine completely replaces asparagine. Glutamine is also a product of the action of dilute acids or barium hydroxide on proteins.

Glutamine forms slender anhydrous needles, soluble in 25 parts of cold water and much more readily at the b. p. It is insoluble in absolute alcohol. The aqueous solution (4 grm. per 100 c.c.) is optically inactive, but the solutions in hydrochloric and oxalic acids are dextrorotatory. -

When heated with alkalies or dilute mineral acids, glutamine yields ammonia and glutamic acid, a body homologous with aspartic acid (page 240).

Glutamine does not reduce Fehling's solution, but dissolves cupric hydroxide to a deep blue solution, a crystallisable compound being formed analogous to that yielded by glycocoll (page 212). Glutamine forms an insoluble compound with mercuric nitrate, a fact utilised by Schulze and Bosshard to isolate it from the juice of beetroot (*Ber.*, **116,** 312; **18,** 290). (See page 239). Glutamine, asparagine, and

other amino-compounds are not precipitated by an acid solution of phospho-tungstic acid.

Glutamic Acid, α-amino-glutaric acid,

$$
\begin{array}{c}
\text{COOH} \\
| \\
\text{CH}_2 \\
| \\
\text{CH}_2 \\
| \\
\text{CH.NH}_2 \\
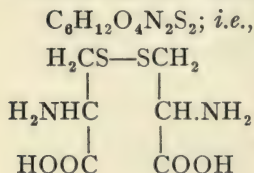| \\
\text{COOH}
\end{array}
$$
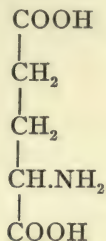
is the higher homologue of aspartic acid, bearing the same relation to normal glutaric acid that aspartic acid bears to succinic acid. Glutamic acid has been isolated from molasses after the sugar has been removed by the strontium process, and is formed, together with aspartic acid, by boiling proteins (*e.g.*, conglutin, maize-fibrin) with dilute sulphuric acid.

Glutamic acid forms orthorhombic tetrahedra, m. p. 202°. It dissolves in about 100 parts of cold water, and is less soluble in alcohol (1 : 500). The solutions are acid, and have an astringent taste, with a peculiar after-taste. The aqueous solution of glutamic acid and of its hydrochloride are dextrorotatory, but its salts with bases are lævorotatory. Glutamic acid differs from aspartic acid in yielding no precipitate with lead acetate even after the addition of ammonia; but the lead salt may be precipitated by adding alcohol to the concentrated filtrate from any precipitate produced by basic lead acetate. Glutamic acid is also distinguished from aspartic acid by not reducing Fehling's copper solution on heating. It forms a characteristic copper salt which is very sparingly soluble in cold water. This fact may be used for the isolation of glutamic acid.

Glutamic acid rotates the plane of polarised light to the right, in the case of the naturally occurring acid obtained through the action of acid; in water $[\alpha]_D = +12°$, but much increased by the presence of acid, thus in 10 % hydrochloric acid $[\alpha]_D = +31°$.

The crude glutamic acid obtained as previously described, is purified by recrystallisation from hot hydrochloric acid. The α-naphthyl-iso-cyanate forms long thread-like needles, m. p. 236°. Benzoyl-*d*. glutamic acid forms plates, m. p. 139°.

Cystin.   Dithio-diamino-dilactic Acid.

$$C_6H_{12}O_4N_2S_2; \textit{i.e.,}$$

$$H_2CS\text{—}SCH_2$$
$$|\qquad\quad|$$
$$H_2NHC\qquad CH.NH_2$$
$$|\qquad\quad|$$
$$HOOC\qquad COOH$$

Cystin is the leading constituent of rarely-occurring urinary and renal calculi. It is also met with as a sediment from urine. It may be prepared from such sediment, or preferably from the calculus when obtainable, by treating the substance with ammonia, and allowing the filtered liquid to evaporate spontaneously, when the cystin is deposited in characteristic colourless or pale yellow hexagonal

FIG. 10.—CYSTIN.

tables of sharp contour (Fig. 10), which are often superposed. Cystin is present in all true and complete proteins, the incomplete proteins, like protamine and fibroin, lack it.

Cystin is colourless, odourless, and tasteless. When heated, it ignites without melting, and burns with a greenish-blue flame, emitting a characteristic penetrating odour resembling that of hydrocyanic acid. Heated in a closed tube, cystin gives off ammonia and yields a distillate of disagreeable odour, leaving a residue of carbon.

Cystin is neutral to litmus, and quite insoluble in water, alcohol, and ether. It is readily soluble in ammonia (distinction from uric acid), in fixed alkali hydroxide and alkaline carbonates, but not in ammonium carbonate. It is precipitated from these solutions by acetic acid. Cystin dissolves in mineral acids and in oxalic acid, but not in tartaric or acetic acid. The solution in hydrochloric acid containing 11.2 % of HCl has a specific rotation of $[\alpha]_D = -224°$.

Cystin forms unstable salts with acids, and is precipitated from their solutions by ammonium carbonate. The hydrochloride unites with mercuric chloride to form a crystalline compound which is nearly insoluble in water.

If a cold solution of cystin in ammonia be treated with ammonio-nitrate of silver, and the liquid then cautiously neutralised by nitric acid, a canary-yellow precipitate is thrown down, but if the solution be heated silver sulphide is precipitated.

When treated with granulated tin in hydrochloric acid solution, cystin is reduced to aminothiolactic acid or cysteïn, $C_3H_7O_2NS$:

$$\begin{array}{l} CH_2SH \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$
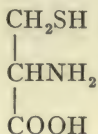
Cysteïn is a crystalline powder, soluble in water, in ammonia, and in acids. The aqueous solution is oxidised to cystin on exposure to the air.

By reaction with nitrous acid cysteïn yields pyruvic acid, $CH_3.CO.COOH$.

When heated with nitric acid, cystin is decomposed with production of a brown colouration.

When boiled with alkali hydroxides, cystin evolves ammonia. The solution then contains a sulphide, and hence gives a black precipitate on addition of lead acetate. The sulphur is not wholly converted into sulphide, even after many hours boiling with alkali hydroxide.

For the detection of cystin in a calculus, the powdered substance should be dissolved in alkali hydroxide, and acetic acid added to the hot solution, when cystin, if present, will separate on cooling, and can be recognised by its crystalline form. Or the calculus may be treated with hot ammonia, and the filtered liquid evaporated to the crystallising point. Any xanthine will be dissolved out and deposited with the cystin.

Goldmann and Baumann (*Zeits. physiol. Chem.*, 1888, 254) have proved that cystin or an allied substance is always present in urine This method is based on the fact that when a few drops of benzoyl chloride are added to a solution of cystin in sodium hydroxide a voluminous precipitate of shining plates of the sodium salt of benzoylcystin, $C_6H_{10}O_4N_2S_2Bz_2$, is formed. This compound is soluble in hot water, less soluble in cold, and quite insoluble when excess of sodium hydroxide is present. On adding a strong acid to the dilute solution, the liquid sets to a transparent jelly, but on warming and standing free benzoyl-cystin separates in dense flocks which can be separated by filtration. Benzoyl-cystin is a strong acid, almost insoluble in water, and but slightly soluble in pure ether, but more readily in ether containing alcohol. In alcohol it dissolves, and crystallises from the solution in slender needles which tend to aggregate in cauliflower-like masses.

Benzoyl-cystin melts at 156–158°. By heating with strong hydrochloric acid, it is decomposed into benzoic acid and cystin. When boiled with sodium hydroxide and lead acetate, it yields black lead sulphide, but the decomposition is not complete even after prolonged boiling.
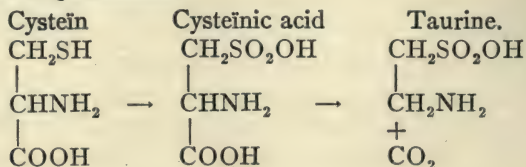
For the isolation of cystin from urine, Goldmann and Baumann recommend that 200 c.c. of the sample should be treated with 10 c.c. of benzoyl chloride and 70 c.c. of sodium hydroxide solution of 1.12 sp. gr., and the mixture shaken until the benzoyl chloride has dissolved. The precipitate (which consists of benzoyl compounds of urinary carbohydrates, mixed with phosphates) is filtered off, and the filtrate rendered strongly acid with sulphuric acid, and shaken with ether containing alcohol. The ethereal layer is separated, evaporated, and the residue boiled for 1 hour with sodium hydroxide and lead acetate. The lead sulphide produced is equivalent to about two-thirds of the cystin isolated; the cystin represented being three-fourths of the actual weight of PbS obtained. From 200 c.c. of normal urine, Goldmann and Baumann obtained 0.0025 grm. of lead sulphide, representing 0.0009 of cystin, for 100 c.c. of urine. In idiopathic cystinuria large amounts of cystin are found in the urine, independent of any formation of calculi, as the result of a metabolic anomaly.

The crude cystin recovered in the method previously described should be purified after removal of the tyrosine by recrystallisation from hot alkaline solution and acidification with acetic acid. If it will not crystallise, it may be purified by precipitation with benzoyl chloride. The benzoylcystin melts at 180°. The phenyl-iso-cyanate forms short clumped needles that melt at 160°; after boiling with hydrochloric acid, the hydantoin is formed that melts at 120°.

Taurine. Aminoethane-sulphonic Acid.

$$C_2H_7O_3NS; \text{ or}$$
$$CH_2.SO_2.OH$$
$$|$$
$$CH_2NH_2$$
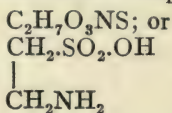
The taurine of the animal body is derived from cysteïn in accordance with the following reactions:

Cysteïn	Cysteïnic acid	Taurine.
CH_2SH	CH_2SO_2OH	CH_2SO_2OH
$|$	$|$	$|$
$CHNH_2$ $\rightarrow$	$CHNH_2$ $\rightarrow$	CH_2NH_2
$|$	$|$	$+$
$COOH$	$COOH$	CO_2

The carbon dioxide is split off, the total action thus constituting a reduction. Taurine is formed in the animal body only in the liver, and varies widely in the bile of different animals.

Minute quantities of taurine are stated to exist in the juices of the lungs and of muscles, but its principal mode of occurrence is in the form of taurocholic acid, $C_{26}H_{45}O_7NS$, which is a characteristic constituent of the bile.[1]

Taurine is best prepared by boiling ox-bile for some hours with dilute hydrochloric acid, separating the liquid from the resinous product, and precipitating the remaining traces of bile-acids by lead acetate. The fitrate is freed from lead by hydrogen sulphide, concentrated, and the taurine which separates on cooling purified by recrystallisation from water.

Taurine has been obtained synthetically by the following series of changes: Ethylene, C_2H_4, is absorbed by fuming sulphuric acid, the product dissolved in water, neutralised by ammonia, and the solution evaporated to the crystallising point. The resultant ammonium isethionate, $C_2H_4O.SO_3(NH_4)$, when heated to 220° yields taurine and water. Taurine has also been obtained by converting ethylene into glycol-chlorhydrin, and treating that substance in the following manner:

Glycol-chlorhydrin. . . . . $HO.C_2H_4.Cl$, heated with K_2SO_3, gives

Potassium isethionate. . . . . . $HO.C_2H_4SO_2.OK$; which, distilled with PCl_5, yields

Isethionic chloride. . . . . $Cl.C_2H_4.SO_2.Cl$; which, on heating with water, yields

Chlorethyl-sulphonic acid . . $Cl.C_2H_4.SO_2.OH$; which, with ammonia at 100° under pressure, gives

Taurine, ... $NH_2.C_2H_4.SO_2.OH$

Taurine crystallises in hard, six-sided prisms (Fig. 11), which crackle between the teeth. It melts at 240° with intumescence and evolution of sulphur dioxide, etc., leaving a difficultly combustible carbonaceous residue.

Taurine has a fresh taste, and is readily soluble in water, but is only

[1] Free taurine was found by Gorup-Besanez in the liver of a person who died from *arachnitis*. It has been detected in the liver in cases of jaundice, and has also been met with in the kidneys, lungs, and muscles. It is likewise present in the intestinal canal and in excrement, doubtless as a product of the decomposition of taurocholic acid.

sparingly soluble in 5% alcohol (1 : 500), and is practically insoluble in absolute alcohol and ether.

Taurine has no acid reaction, but it forms soluble crystallisable salts with bases. It dissolves in hot dilute acids, and separates unchanged on cooling. When the solution of taurine is evaporated with alkali hydroxide, the whole of the nitrogen is evolved as ammonia, and the residue contains a sulphite and acetate of alkali-metal. Fused with potassium hydroxide, the same products are obtained. If heated strongly with sodium carbonate, avoiding access of air, taurine yields a product containing much sodium sulphide. Hence the solution of the mass in water blackens a silver coin, and evolves hydrogen sulphide when treated with an acid.

By reaction with nitrous acid, taurine yields isethionic acid.
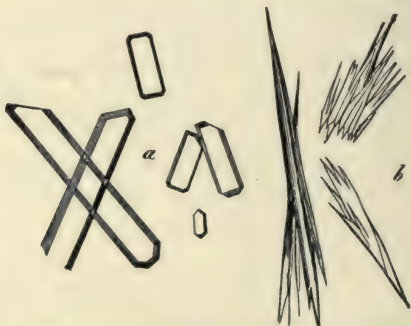
FIG. 11.—Taurine. a, well-formed six-sided prisms; b, irregular sheaf-like masses from an impure solution.

Solutions of taurine are not precipitated by metallic salts nor by tannin.

For the demonstration or isolation of taurine, in bile or tissue, the material should be boiled with 10% hydrochloric acid for 6 hours, and the hot solution filtered through animal charcoal. It is then concentrated to a small volume and again filtered hot to remove salts. The filtrate is then evaporated to dryness, the residue dissolved in 5% hydrochloric acid and thetaurine precipitated by the addition of 10 volumes of alcohol. The crystals should be purified by recrystallisation from hot water to which hot alcohol has been added. Taurine combines with mercuric oxide to form a white compound insoluble in the hot mixture. The sulphur content is the basis of the quantitative estimation.

Alanine. α–Amino-propionic Acid.

$$C_3H_7O_2N =$$
$$CH_3$$
$$|$$
$$CHNH_2$$
$$|$$
$$COOH$$
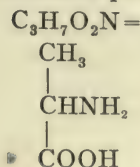
Alanine occurs in the dextrorotatory form in nearly all animal and vegetable proteins, the albuminoids being especially rich in it. It has a sweetish taste, is very soluble in water, quite insoluble in alcohol. The specific rotation is $[\alpha]_D + 2.7°$ in aqueous solution, but $+ 10°$ in dilute hydrochloric acid. It dissolves cupric hydroxide on boiling, with the production of a soluble crystalline copper salt. It occurs mixed with leucine and valine in the distillate by the ester method, and must be separated from these by fractional crystallisation. Characteristic derivatives are the β-naphthalene-sulpho-d-alanine (fine clustered needles that melt at 81°), the phenyl-iso-cyanate (needles that melt at 168°) and the naphthyl-iso-cyanate (small fine needles that melt at 195°).

Valine. α-Amino-valeric Acid.

$$C_5H_{11}NO_2 =$$
$$CH_3 \quad CH_3$$
$$\diagdown \diagup$$
$$CH$$
$$|$$
$$CHNH_2$$
$$|$$
$$COOH$$
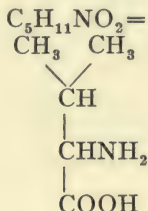
Valine occurs in traces in most proteins, even in the protamines. It is soluble in water, and quite soluble in alcohol, especially methyl alcohol. It has a sweetish taste, with a bitter after-taste. It dissolves cupric hydroxide on boiling, the copper salt is easily soluble in water, tends little to crystallisation and is soluble in methyl alcohol. The naturally occurring valine has $[\alpha]_D = + 28.8°$ in 20 % hydrochloric acid. It is obtained mixed with the crystals of leucine, from which it must be separated by fractional crystallisation. It forms no really well characterised derivative; the hydantoin of the phenyl-iso-cyanate melts at 131°.

Serine.   α-Amino-β-hydroxypropionic Acid.

$$C_3H_7O_3N=$$
$$CH_2OH$$
$$|$$
$$CHNH_2$$
$$|$$
$$COOH$$
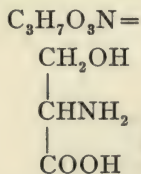
Serine exists widespread in animal and vegetable proteins, in amounts larger then usually reported, on account of the difficulties in the quantitative isolation. The naturally occurring form is usually racemic. It has been prepared synthetically by Fischer and Leuchs by the reaction between ammonia, hydrocyanic acid and glycollic aldehyde, and the *d-* and *l-*serine may be obtained from the racemic amino-acid. Serine is obviously derived in nature from alanine, and on reduction yields alanine. It is the amine of glyceric acid. Serine is rather soluble in water, quite soluble in alcohol. It has a peculiar sweetish taste, with an unpleasant after-taste. In the ester method it is obtained as the final amino-acid in the fraction distilling over at the highest temperature. It is difficult to crystallise out. The β-naphthalene-sulpho-serine tends to be amorphous, but on repeated crystallisation from hot alcohol forms small needles that melt at 214°. The phenyl-iso-cyanate-serine forms fine, clustered needles, soluble in both water and alcohol, melting at 168°.

Tryptophane.   Indole-α-amino-propionic Acid.

$$C_{11}H_{12}O_2N_2=$$
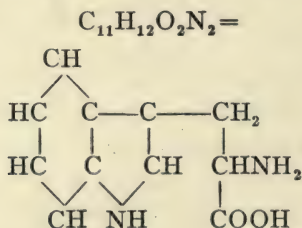
Tryptophane exists, in small quantities, in nearly all proteins, the keratins and collagens and the protamines being devoid of it. It is an amino-acid of the greatest importance in metabolism, and no protein devoid of it can be a complete protein in tissue-building. It is the mother substance of the indole, that as indole and skatole and

indican and skatoxyl appear in the fæces and urine. The processes of digestion simply split off the tryptophane; it is bacterial processes that split this molecule and set the indole free. It has been synthesised by Ellinger and Flammand (*Ber.*, 1907, **40**, 3,029). The β-indole-aldehyde is first condensed with hippuric acid to form the lactimide, which on being boiled with dilute alkali hydroxide takes up water and yields indoxyl-α-benzoyl-amino-acrylic acid. The benzoyl group is then split off by the action of sodium ethoxide, following which reduction yields tryptophane. The naturally occurring tryptophane is the l-form, $[\alpha]_D = -30°$. In hydrochloric acid and in potassium hydroxide, it is dextrorotatory, and the racemic compound is easily formed. Tryptophane has a bitter taste, it is quite soluble in even cold water, almost insoluble in alcohol. It melts at about 255°, when slowly heated.

The chemical processes necessary for the isolation of tryptophane are complicated and not wholly satisfactory. If it is to be sought for in a protein, the protein must be digested with ferment, not hydrolysed with acid. The protein is to be suspended in water, trypsin added, the solution made alkaline, toluene added and the mass allowed to stand for a week or more. From time to time the bromine test may be applied, and from this one may judge when the yield is complete. Finally the mass is heated to 80°, filtered, sulphuric acid added to make a concentration of 5%, and a 10% solution of mercuric sulphate in 5% sulphuric acid added in excess. The heavy precipitate is collected on a filter, washed with 5% sulphuric acid with the aid of suction, suspended in water, heated and the mercury precipitated with hydrogen sulphide. The mixture is filtered and the hydrogen sulphide expelled with carbon dioxide. Sulphuric acid is again added to make 5%, and a solution of mercuric sulphate carefully added until a clinging precipitate is formed that does not seem to increase. This consists of cystin and other impurities, and should be removed by filtration. To the filtrate is then added excess of the mercuric sulphate, the precipitate collected upon a Buchner funnel and washed with 5% sulphuric acid until the wash water gives no reaction with Millon's reagent. The precipitate is then suspended in water, the mercury precipitated from the hot solution with hydrogen sulphide, filtered, the sulphuric acid removed with barium hydroxide, and the final filtrate concentrated at 40°, at diminished pressure. A little alcohol is then added, and the concentration continued under diminished pressure at

40°, until signs of crystallisation appear. After standing on ice for several days, the crystals are suspended in warm water and recrystallised from 50% alcohol. One recrystallisation may be necessary. If preformed tryptophane be sought for in a material, one would begin where the first precipitation with mercuric sulphate is effected. It crystallises in soft, 6-sided crystals.

When a solution of tryptophane is mixed with bromine water, a rose-red colour develops. A pine stick moistened with hydrochloric acid and then washed off with water is immersed in a solution of tryptophane; on drying, the purple colour of the pyrrole reaction develops. If an aqueous solution of glyoxylic acid be mixed with a solution of tryptophane and strong sulphuric acid then poured down upon the mixed solution, a red-violet colour will appear at the contact of the two layers (Hopkins and Cole, *Proc. Royal Soc.*, **68**). When heated, indole and skatole are formed; many bacteria also form indole from a culture medium containing tryptophane. β-naphthalene-sulpho-tryptophane forms fine needles, m. p. 180°; the α-naphthyl-iso-cyanate-tryptophane melts at 158°; the benzene-sulpho-tryptophane forms small leaves that melt at 185°.

When tryptophane is putrified by aerobic bacteria, indole and skatole are formed. When putrified by anaerobic bacteria, indole-propionic and indole-acetic acid are formed. The relations in the human intestine are complex, and there is evidence that both these reactions occur normally. A fraction of the bacterial derivatives are resorbed from the intestine (from the colon particularly, it is there that the bacterial reactions largely occur) and are the source of the indole substances in the urine.

Indole. C_8H_7N; *i.e.,*

$$
\begin{array}{ccc}
& \text{CH} & \\
& \diagup \diagdown & \\
\text{HC} & \text{C}\!-\!\!-\!\!-\!\text{CH} & \\
| & | & | \\
\text{HC} & \text{C} & \text{CH} \\
& \diagdown \diagup \diagdown \diagup & \\
& \text{CH} \quad \text{NH} &
\end{array}
$$
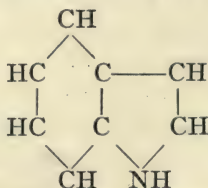
Indole is so named from being the nucleus from which the indigo-group of chemical compounds is derived. It has been obtained synthetically by several reactions, and is, together with skatole, a characteristic constituent of fœces. Its occurrence therein is due to the

putrefactive decomposition of tryptophane which usually takes place to a greater or less extent in the intestinal canal, especially in the colon. The greater part of the skatole and indole formed is eliminated by the kidneys in the forms of skatoxyl- and indoxyl-sulphuric acids (the so-called "urinary indican"), the remainder being excreted in the fœces; the fractions are, however, variable.

Indole is formed when albuminous substances are fused with potassium hydroxide but it is more easily obtained by digesting liver or fibrin with water and pancreatin. Following digestion, putrefaction is allowed to occur. If the digestion be not too prolonged, the product when acidified and distilled yields a distillate from which impure indole can be extracted by agitation with ether. On evaporation, the ether leaves a residue of indole mixed with skatole and phenol. From the last body indole can be purified by dissolving it in benzene and adding a benzene solution of picric acid, when indole picrate, $C_8H_7N,C_6H_3(NO_2)_3OH$, crystallises out in long red needles, which are sparingly soluble in cold benzene, readily in hot, very slightly soluble in petroleum-spirit, and decomposed by ammonia.

From skatole, indole may be separated by dissolving the mixture in the smallest possible quantity of absolute alcohol, and then adding from 8 to 10 volumes of water, when the skatole will be precipitated while the indole remains in solution.

When pure, indole forms crystalline scales of a satiny lustre. It has a persistent and disgusting fœcal odour, melts at 52°, and boils with partial decomposition at about 245°. Indole distils readily in a current of steam. It is very soluble in alcohol, ether, and petroleum-spirit, and dissolves with moderate facility in hot water, separating on cooling in oily drops, which subsequently form plates resembling benzoic acid.

Indole possesses feeble basic characters. When treated with strong hydrochloric acid it forms a sparingly soluble salt which is decomposed by boiling with water. The picrate has already been described.

If an aqueous solution of indole be treated with fuming nitric acid, or preferably with a solution of sodium nitrite acidified with sulphuric or nitric acid, a red precipitate is formed of the composition $C_{16}H_{13}(NO)N_2,HNO_3$. A deep red colouration is produced when an alcoholic solution of indole is treated with nitrous acid, or nitrogen trioxide passed through it.

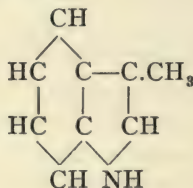If a strip of pine wood be moistened with strong hydrochloric acid

and immersed in an alcoholic solution of indole, or exposed to the vapours of indole, it is coloured deep crimson.

When a dilute solution of indole is treated with sodium nitroprusside, and a few drops of sodium hydroxide solution added, a violet-blue colouration is produced, which changes to pure blue when the liquid is acidified with acetic acid.

On melting a minute quantity of indole in a test-tube with dehydrated oxalic acid, a fine magenta colouration is produced. The colouring matter formed is soluble in acetic acid. Indole mixed with a few drops of a 5 % solution of formaldehyde and strong sulphuric acid yields a violet colour; skatole gives a yellowish-brown.

Indole is decomposed when boiled with moderately concentrated sodium hydroxide, which behaviour distinguishes it from skatole.

Skatole, or Methyl-indole,

$$\begin{array}{c}
\text{CH} \\
\diagup\diagdown \\
\text{HC} \quad \text{C---C.CH}_3 \\
\mid \qquad \mid \qquad \mid \\
\text{HC} \quad \text{C} \quad \text{CH} \\
\diagdown\diagup\diagdown\diagup \\
\text{CH} \quad \text{NH}
\end{array}$$

closely resembles indole. Its odour is somewhat similar and equally persistent and unpleasant. It crystallises from hot water or, preferably, petroleum-spirit in glittering white scales, melts at 95°, and boils at 265°.

Skatole gives no colour-reactions with pine wood and hydrochloric acid, nor with sodium nitroprusside, and also differs from indole in not suffering decomposition when boiled with moderately concentrated sodium hydroxide. The picrate is precipitated in red needles on mixing hot aqueous solutions of skatole and picric acid. When sodium nitrite is added to a solution of skatole in glacial acetic acid, a dark brown colouration is produced, and on adding water the nitrosamine is precipitated as a yellow oil, which solidifies in a freezing mixture to a crystalline mass. E. Fischer describes the formation of this compound as highly characteristic, and available for the detection of skatole and its separation from indole. When the picrates of indole and skatole are distilled with ammonia, both pass over; if they are distilled with sodium hydroxide, the skatole passes over, the indole is decomposed.

Indoxyl,

$$\begin{array}{ccc}
 & CH & \\
 & \diagup\diagdown & \\
HC & C\!-\!C.OH & \\
| & | \quad | & \\
HC & C \quad CH & \\
 & \diagdown\diagup\diagdown\diagup & \\
 & CH \quad NH &
\end{array}$$
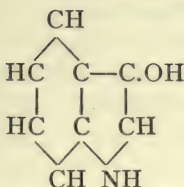
is interesting as a substance intermediate between indole and indigotin. It possesses both basic and acid characters. Its alkaline solution absorbs atmospheric oxygen with formation of indigo-blue, which is also produced on adding ferric chloride to a hydrochloric acid solution of indoxyl.

Indoxyl-sulphuric Acid, $C_8H_5(NH).SO_4H$, occurs in urine, largely as a potassium salt. The normal excretion contains only traces of this compound, which has received the name of "urinary indican," from a supposed identity with plant-indican, the glucoside from which indigo is obtained. The only similarity between the two substances is that both yield indigo-blue as one of the products of their decomposition.[1]

Potassium indoxyl-sulphate, $C_8H_5(NH).SO_4K$, crystallises from hot alcohol in colourless lustrous tables, readily soluble in water but only sparingly in cold alcohol. When boiled with dilute acid it is decomposed into indoxyl and acid potassium sulphate, but is not attacked by alkalies. When the crystals are heated, indigotin (indigo-blue) sublimes, and the same substance is formed quantitatively when the acidified solution is warmed with ferric chloride.

For the detection of indoxyl-sulphuric acid in urine, Jaffé (*Pflüger's Archiv.*, **3,** 448) first separates any albumin by boiling the liquid, and treats the filtrate with an equal volume of hydrochloric acid. A dilute solution of bleaching power is then cautiously added, until the blue colour no longer increases. On agitating with chloroform the colouring matter is taken up and can be obtained on evaporation. Jaffé's method is not suitable for the detection of traces of indigogen, as the colouring matter is destroyed by the least excess of the oxidising agent. Hence MacMunn boils the urine with an equal volume of hydrochloric acid and a few drops of nitric acid, cools, and agitates with chloroform. The chloroform is generally coloured

---

[1] Decomposing urine occasionally forms a bluish-red pellicle, and ultimately deposits microscopic crystals of indigo-blue. A calculus of the same nature has been described.

violet, and, when examined in the spectroscope, shows two broad absorption-bands, one on either side of the D line. The less refrangible is due to indigo-blue and the more refrangible to indigo-red; though it is doubtful if the latter colouring matter is identical with the indirubin which occurs in commercial indigo.[1]

A. C. Méhu (*J. Pharm.*, [v], **7,** 122) adds to the urine about 0.5 c.c. of strong sulphuric acid to 1 litre of the sample, and then saturates the liquid with powdered ammonium sulphate, whereby any indigotin or indirubin is precipitated. On treating the precipitate in the cold with 50% alcohol the indirubin will be dissolved, while the insoluble indigotin is purified by washing with water, followed by spontaneous drying. Méhu proposes a colourimetric process for the estimation of indigotin, which he dissolves in hot carbolic acid to which sufficient glycerin or absolute alcohol has been added to prevent crystallisation on cooling. The colour of a solution of indigo-blue of known strength, prepared in this manner, is compared with that of the urinary pigment. Isatin reacts with indican to form two molecules of indigo-red. The reagent is prepared by dissolving 25 mgrm. of isatin in 100 c.c. of strong hydrochloric acid. Equal parts of this reagent and urine are simmered for a few moments, and then extracted with chloroform. Normal urine will only impart a faint pink to the layer of chloroform, abnormal urine may yield as much colour with a dilution of 1:1,000.

W. Michailoff (*J. Chem. Soc.*, **54,** 880) also saturates the acidified urine with finely powdered ammonium sulphate, and then extracts the urobilin by repeated agitations with ethyl acetate. The aqueous layer is next mixed with an equal volume of fuming hydrochloric acid, chloroform added, and then cautiously treated with dilute bromine-water, agitating well between each addition. By presenting the indigo with the solvent when in the nascent state its extraction is said to be very readily and perfectly effected.

Indoxyl-sulphuric acid occurs in very small quantities in normal

[1] For the detection of indirubin, O. Rosenbach (*Jour. Chem. Soc.*, **58,** 1032) adds nitric acid to the boiling urine, cools, adds a large excess of ammonia, and agitates with ether, which will acquire a purple colour if indirubin be present. For its isolation, Rosenbach treats the fresh urine with lead acetate, heats the filtered liquid to boiling, and adds nitric acid, drop by drop, until a purple colour is produced, carefully avoiding excess of acid. The liquid is then cooled and treated with ammonia till alkaline. The precipitate is filtered off, washed in succession with ammonia, dilute hydrochloric acid, and water, and then dissolved in boiling alcohol. The solution deposits indigo-blue on cooling. It is filtered and the filtrate treated with alcoholic lead acetate, again filtered, and most of the alcohol boiled off. On diluting the residual liquid with water, impure indirubin is precipitated as a brown powder, which, after washing with water, may be purified by crystallisation from chloroform or ether.

human urine, Jaffé finding from .004 to 0.019 grm. in 1,500 c.c. of the excretion. Horse's urine contains twenty times as much. The proportion in human urine is much increased in certain diseases, such as cholera, typhus, peritonitis, dysentery, and Addison's disease. In obstructive diseases of the small intestine the increase is enormous. The presence of a large amount of indigogens in the urine generally implies that abundant albuminous putrefaction is in progress in some part of the system, these putrefactive products being absorbed and eliminated by the kidneys in the forms of indoxyl-sulphuric acid and its analogue skatoxyl-sulphuric acid, $C_8H_6(CH_3)N.SO_4H$. The latter substance is said to be somewhat more abundant in human urine than the indoxyl-compound. When decomposed by hydrochloric acid or an oxidising agent, it gives a colouring matter usually reddish, but which may possess a marked purple tint.

Traces of compounds of indoxyl and skatoxyl exist in normal urine, with glycuronic acid and their proportions appear to be greatly increased under certain conditions.

The quantitative estimation of indoxyl in urine or other material is difficult and unsatisfactory. The material is first acidified with acetic acid and distilled. The distillate is then made alkaline and again distilled, the indole and skatole passing over while the phenols are retained. The distillate is then acidified with hydrochloric acid, and the substances precipitated as picrates. The picrates are then distilled with ammonia, and the indole and skatole removed from the distillate by repeated extractions with ether. The ethereal extracts are evaporated, and the residue dissolved in a small amount of warm water and 10 volumes of alcohol added; indole remains in solution, skatole is precipitated. The filtrate is then taken up in water, and in a known fraction oxidation to indigo-blue or indigo-red is accomplished, and the colour compared with a standard of indigo in a colourimeter. A more accurate method would be to titrate the indigo-red or blue with a standard solution of potassium permanganate.

Proline. *α*-Pyrrolidine-carboxylic Acid.

$$C_5H_9\ O_2N=$$

$$\begin{array}{c} H_2C\text{——}CH_2 \\ |\qquad\quad| \\ H_2C\qquad CH.COOH \\ \diagdown\ \diagup \\ NH \end{array}$$
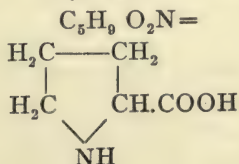
Recent investigations of the Fischer school have taught us that nearly all proteins contain proline, and it is apparently an essential component of the true or complete proteins. Pyrrole is otherwise of importance in the body; mention has already been made of tryptophane, and among the other pyrroles in the body are hæmin, hæmatoporphyrin, bilirubin, urobilin and urochrome. Proline is not present in large amounts in the higher proteins. Proline is present in some protamines to as much as 13 %. Its isolation in the course of the ester method (see page 218), has been described. The impure material is purified by the formation of the copper salt, the removal of the copper by hydrogen sulphide, followed by hot filtration through animal charcoal. Proline is soluble in alcohol as well as water, but the recrystallisation is best done from hot alcohol. The natural proline is both *l*- and racemic proline. The specific rotation of the *l*-proline is $[\alpha]_D = -77°$. Proline is precipitated by phosphotungstic acid in sulphuric acid solution. Oxyproline often accompanies proline. The proline crystallises in flat needles, that do not have a definite m. p. β-Naphthalene-sulpho-*l*-proline crystallises in long flat needles that melt at 138°, and are soluble in alcohol. Phenyl-iso-cyanate-proline forms resinous needles; when concentrated in 5 % hydrochloric acid, the anhydride is formed which crystallises in long flat needles, m. p. 144°. Picrates and picrolonates are also formed.

Phenylalanine.   Phenyl-α-amino-propionic Acid.

$C_9H_{11}NO_2$:

$$
\begin{array}{c}
CH \\
HC \diagup \diagdown CH \\
| \qquad | \\
HC \qquad CH \\
\diagdown \diagup \\
C \\
| \\
CH_2 \\
| \\
CHNH_2 \\
| \\
COOH.
\end{array}
$$
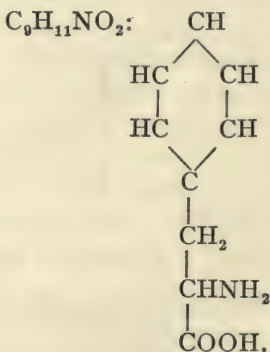
This is a direct derivative of alanine and is closely related to tyrosine. It is present in all the higher or complete proteins, and seems to be most often centrally located in the nucleus of the molecule, since it is recovered late in the course of the hydrolysis of a protein. It is of great importance physiologically, though it is possible that it and tyrosine

are reciprocal in the anabolic processes of the protein metabolism. The naturally occurring form rotates the plane of polarised light to the left, $[\alpha]_D = -35°$. It is not very soluble in cold water, and is quite insoluble in alcohol. Phenylalanine has a bitterish taste. The crude phenylalanine, isolated as previously described, is purified by recrystallisation from hot water. The β-naphthalene-sulpho-phenylalanine crystallises in very long fine needles, m. p. 143°. The phenyl-iso-cyanate-phenylalanine forms fine colourless needles that are almost insoluble in cold water, m. p. 200°.

From tyrosine and phenylalanine are derived the phenol and cresol substances that are present in the urine. In the intestine these two amino-acids are subjected to bacterial putrefaction, and as the reactions are not completed, we have in the urine several aromatic substances that are derived from this intestinal decomposition. The list of aromatic substances includes the following. Phenol-sulphuric acid; cresol-sulphuric acid; pyrocatechin and quinol, combined with sulphuric acid; p-hydroxy-phenyl-lactic acid and p-hydroxy-phenyl-propionic acid; and probably dihydroxy-phenyl-lactic acid. In these conjugations of the aromatic substances the sulphuric acid may be substituted by glucuronic acid. In the metabolic anomaly known as alkaptonuria, we have in the urine an abnormal aromatic body, homogentisic acid (dihydroxy-phenyl-lactic acid) supposed to be a normal intermediary stage that appears as an end product in this condition.

Histidine. α-Amino-β-iminazolyl-propionic Acid.

$$C_6H_9O_2N_3 \;=\; \begin{array}{l} \text{CH—NH} \\ \;\parallel\quad\;\;\;\rangle\text{CH} \\ \text{C—N} \\ \;\mid \\ \text{CH}_2 \\ \;\mid \\ \text{CHNH}_2 \\ \;\mid \\ \text{COOH} \end{array}$$
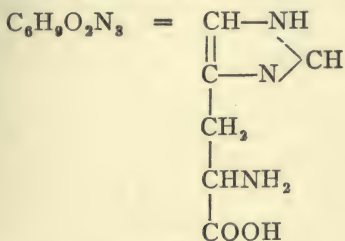
Histidine is contained in nearly all complete proteins, and in some of the simpler protamines. Globin is especially rich in histidine. Histidine is a base, very soluble in water, quite soluble in alcohol, and rotates the plane of polarised light to the left, $[\alpha]_D = -38°$. When recovered in the process of isolation, it is in solution. It is purified by

precipitation by the addition of a solution of mercuric sulphate acidi-
fied with sulphuric acid, the precipitate washed, the mercury removed
by hydrogen sulphide, the filtrate freed of sulphuric acid by barium
hydroxide, the excess of barium removed with carbon dioxide, the final
filtrate concentrated and hydrochloric acid added until the reaction is
faintly acid.   On concentration and standing, the hydrochloride will
crystallise out in large plates.   The presence of alcohol aids the crystal-
lisation.   When histidine is heated with hydrochloric acid in methyl
alcohol, the methyl ester is formed, whose dihydrochloride is a crystal-
line salt, m. p. 196°.   Histidine responds to the biuret test.   On warm-
ing with bromine water, a red colouration gradually appears, followed
by the deposition of reddish particles.   If a solution of histidine be
made alkaline with carbonate, the addition of diazo-benzene-sulphonic
acid will cause the production of an intense red diazo reaction.   The
most characteristic salt is the picrolonate.   Picrolonic acid in alcoholic
solution is added to the solution of histidine, in slight excess only, and
the mixture set aside for several days for crystallisation.   The crystals
are washed with cold water, and may be dried and weighed as a
quantitative estimation.   The fine yellow needles melt at 220°.

Arginine.—Guanidine-α-amino-valeric acid.   This amino-acid is a
combination of urea with ornithine, according to the equation:

$$
\begin{array}{ccccc}
NH_2 & & CH_2NH_2 & & NH_2 \\
\diagup & & | & & | \\
C=O & & CH_2 & & HN=C\!-\!\!-\!NH \\
\diagdown & + & CH_2 & = & | \\
NH_2 & & | & & CH_2 \\
& & CHNH_2 & & | \\
& & | & & CH_2 \quad + H_2O \\
& & COOH & & | \\
& & & & CH_2 \\
& & & & | \\
& & & & CHNH_2 \\
& & & & | \\
& & & & COOH
\end{array}
$$
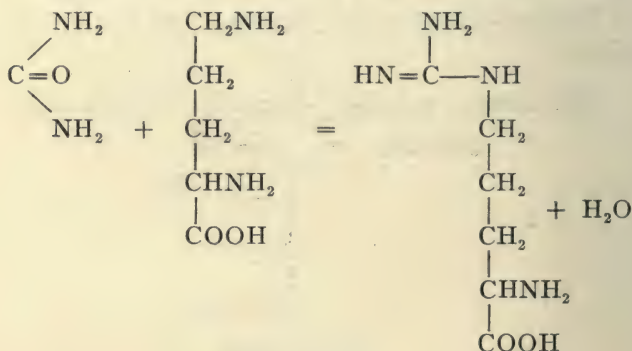
Arginine is a component of nearly all proteins, the simple protamines
being especially rich in it.   It is strongly basic, and very soluble in
water, slightly soluble also in alcohol.   It is dextrorotatory, $[\alpha]_D = +10°$
in hydrochloric acid solution.   It is decomposed by a special ferment
of the liver, arginase, with the production of urea and ornithine.   It

may be prepared synthetically from ornithine and cyanamide. It forms both simple and double salts. The combinations with silver used for its isolation, and with copper nitrate are quite insoluble. The β-naphthalene-sulpho-arginine forms light powdery crystals, the m. p. being 87°. Dibenzoyl-arginine crystallises in long plate-like needles that melt at 218°. There is a picrate, but the most characteristic salt is the picrolonate, which forms yellow needles that melt at 110°, and are very insoluble in both water and alcohol. A quantitative estimation is best made by weighing the picrolonate.

Lysine.   α-ε-Diamino-caproic acid.

$$C_6H_{14}O_2N_2 \;=\; \begin{array}{l} CH_2NH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CH_2 \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$
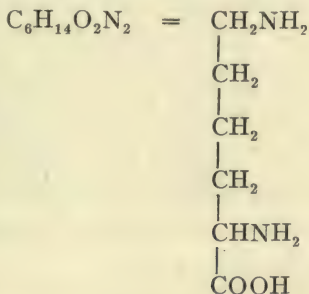
Lysine is a base widely present in both animal and plant proteins. It is very soluble in water, and does not crystallise as such. The naturally occurring form is dextrorotatory, $[\alpha]_D = 14$ to 17., depending upon the relations of concentration. Heating with barium converts it into the racemic state. Lysine is subject to bacterial putrefaction and is the parent substance of penta-methylene-diamine. It forms acid and double salts, *i.e.*, with silver nitrate one salt will have included a molecule of nitric acid, the other not. The lysine isolated as previously described (see page 223) is best purified by precipitation with picric acid. To the solution of lysine, concentrated almost to dryness, an alcoholic solution of picric acid is added so long as a precipitate forms. After a couple of days the crystals of picrate are filtered off, washed with absolute alcohol, dissolved in a small amount of boiling water, filtered and the filtrate concentrated, following which the crystallisation will again occur on cooling. The crystals may then be washed in absolute alcohol and when weighed give a fair result for a quantitative estimation. To recover the lysine, the crystals of picrate are

dissolved in hot 5 % sulphuric acid, the picric acid removed by shaking
with ether and the lysine precipitated with phosphotungstic acid as
previously described, and again isolated as before. The phenyl-iso-
cyanate forms when boiled with hydrochloric acid a hydantoin that
melts at 183°.

Quantitative Estimation of Monamino-acids.

This may now be accomplished in one of two ways. If the total
amino-acids are to be estimated, the histidine, lysine and arginine may
be separated as previously described and estimated by the Kjeldahl
method. For the estimation of the monamino-acids, the material
(urine, culture medium, tissue extract, etc.) is suspended in hot water
and filtered. The cooled filtrate is then acidified with sulphuric
acid to a 5 % concentration, and then precipitated with phospho-
tungstic acid, 10 % solution in 5 % sulphuric acid. The precipitate
is collected upon a Buchner funnel, thoroughly washed with 5 %
sulphuric acid, the excess of the reagent and the sulphuric acid
removed with barium hydroxide and the excess of the barium removed
by the careful addition of sulphuric acid or of carbon dioxide. In the
final filtrate are the amino-acids.

The method of Sörensen (*Biochem. Zeitschrift*, 1908, **7**, 45 and 407)
is based upon the previously stated fact that formaldehyde combines
with the NH_2 groups of amino-acids, and from the estimation of the
carboxyl groups, the quantity of the amino-acid may be calculated.
An ammonia estimation must be made separately, as this is to be sub-
tracted. 50 c.c. of the material to be tested is placed in a 100 c.c.
flask, 1 c.c. of a 1 % solution of phenolphthaleïn is added and 2 grm.
crystalline barium chloride. After the salt has dissolved, a saturated
solution of barium hydroxide is added until the reaction is alkaline, and
then 5 c.c. more added, after which the flask is filled to the mark, shaken
and filtered, to remove the phosphates. 80 c.c. of the clear filtrate
are taken (corresponding to 40 c.c. of the original material) and placed
in another 100 c.c. flask, made neutral to litmus paper by the addition
of $N/10$ hydrochloric acid and then diluted to the mark. 40 c.c. are
used for the estimation of ammonia, and the same amount for the
estimation of the amino-acids. To this 40 c.c. is added a solution of
formaldehyde and titrated as follows. It is necessary to use a freshly
prepared formaldehyde and a control in each test. To 20 c.c. of
water are added 10 c.c. of a solution of formalin to which has been

added 1 c.c. of a solution of 0.5 grm. phenolphthalein in 100 c.c. of 50% alcohol, and enough $N/5$ barium hydroxide to produce the faintest rose colour. 5 c.c. of $N/5$ barium hydrozide solution are then added, and the mixture titrated with $N/5$ hydrochloric acid until a faint rose colour appears, then one more drop is added and the colour becomes a distinct red. The material is then treated in the same way, 10 c.c. of the formalin solution is added, 5 c.c. of the barium hydroxide solution, or more if necessary, and titrated with $N/5$ hydrochloric acid until the same colour is reached as in the control. To the control solution 2 drops of the barium hydroxide solution are then added, with the production of a deep red colour. The test solution is then titrated with further additions of the barium hydroxide solution until the same intensity of colour is produced. Each c.c. of the $N/5$ solution corresponds to 2.8 mgrm. of NH_2 nitrogen, after subtraction of the ammonia estimated in direct test (see also Vol. VIII).

A much more adaptable method of estimation is the new procedure of Van Skyke for the utilisation of a long-known reaction of aminonitrogen with nitrite. The reaction is as follows:

$$RNH_2 + HNO_2 = ROH + H_2O + N_2.$$
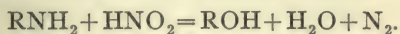
By the measurement of the nitrogen, the estimation may be very accurately accomplished. Since nearly all the natural materials that one might desire to submit to such an analysis contain either urea or ammonia or both, these must first be removed. The material is dissolved or suspended in water and sulphuric acid added to 25% strength, and the mixture heated in an autoclave at 175° for 1 1/2 hours. This converts the urea into ammonia but does not affect the aminoacids. The solution is then transferred to a distillation flask, made alkaline with an excess of calcium hydroxide and distilled until the fumes are free of ammonia. The foaming may be moderated by the addition of a piece of paraffin. The residue is then filtered, the precipitate on the filter carefully washed with hot water, and the combined filtrate then evaporated nearly to dryness. The final solution is then filtered into a 25 c.c. flask, and the filter paper repeatedly washed with small portions of water and the flask filled up to the mark. The final solution should be made acid with sulphuric acid, and for the estimation an amount should be taken containing not over 20 mgrm. of amino-nitrogen. It will be best to describe the method in the words of the author (*J. Biol. Chem.*, 1911, **9,** 186).

Principle of the Method.

Nitrous acid in solution spontaneously decomposes with formation of nitric oxide:

$$2HNO_2 \rightleftarrows HNO_3 + NO.$$

This reaction is utilised in displacing all the air in the apparatus with nitric oxide. The amino solution is then introduced, evolution of nitrogen mixed with nitric oxide resulting. The oxide is absorbed by alkaline permanganate solution, and the pure nitrogen measured in the special gas burette shown in the figure.

Reagents.

The permanganate as absorbent for nitric oxide was chosen after trial of all the solutions recommended in the literature. Ferrous sulphate solution, which is ordinarily recommended in gas analysis methods, is entirely unsatisfactory. The reaction by which ferrous sulphate and nitric oxide combine is reversible, and the nitric oxide in solution attains an equilibrium with that in the supernatant gas. Therefore even approximately complete absorption is possible only with perfectly fresh ferrous sulphate solution, and even with this, is a comparatively slow process. Results become inaccurate before the solution has absorbed its own volume of nitric oxide. Sulphite solution, recommended by Divers, is even less satisfactory. A strong solution of sodium dichromate in sulphuric acid, which oxidises the oxide to nitric acid, is better, but is somewhat viscous. Acid permanganate, unless in very dilute solution, gradually decomposes giving off oxygen, which supersaturates the solution. 1 % permanganate in 1 % sulphuric acid gives accurate results, however, if the solution is freed from excess oxygen by shaking thoroughly with air immediately before use. Alkaline permanganate, originally employed by Hans Meyer, proved an absolutely satisfactory absorbent solution in every respect. It is entirely stable, can be used in concentrated solution, and oxidises the nitric oxide to nitrate with such rapidity that the gas is absorbed about as fast as is carbon dioxide by potassium hydroxide solution. A solution containing 50 grm. of potassium permanganate and 25 grm. of potassium hydroxide per litre was adopted for permanent use. The manganese dioxide formed by reduction is in such a fine state of division that

it does not interfere at all with the use of the solution in a Hempel absorption pipette, and a large number of determinations can be made without changing the solution. In order to prevent deposition of manganese dioxide in the capillaries, it is well to leave G (see Fig. 12) filled w th water from the gas burette, rather than with permanganate, when the apparatus is not in use. As the alkaline solution absorbs carbon dioxide as well as nitrogen, the presence of carbonate in the amino solution does not interfere with the determination.

For decomposing the amino substance the most satisfactory conditions are, a great excess of nitrite, from which the nitrous acid is freed by an equivalent of a weak acid (acetic). The great excess of reagent forces the reaction to rapid completion. The use of a weak acid, instead of the mineral acids employed in previous methods, causes evolution of a relatively small volume of nitric oxide, and avoids danger of acid hydrolysis of the more complex proteolytic products. In dissolving amino substances not readily soluble in water alone, one may use mineral acids of not more than $N/2$ concentration, acetic acid of any concentration up to 50 %, or fixed alkali up to $N/1$ concen-·tration. A few drops of sodium hydroxide solution are usually added to assist in dissolving tyrosine and lysine picrate.

Corrections for Impurity in Reagents.—As commercial sodium nitrite often contains impurities which gradually evolve traces of nitrogen when the nitrite is acidified, each lot of the latter must be tested before it is used, and, a correction for the reagent employed, if necessary, in calculating subsequent results. A typical "C.P." commercial nitrite yielded o.2 c.c. of nitrogen in 5 minutes, o.3 c.c. in 1/2 hour, and o.5 c.c. in 2 hours.

Apparatus.

The apparatus[1] is shown in the figure. The reaction is carried out in D, a bottle of 35–37 c.c. capacity. It is fitted with a 4-hole rubber stopper, which holds permanently the tubes shown in the figure. The stopper is held firmly in place by a strip of picture wire passing through loops of stout copper wire on opposite sides of the neck of the bottle. All the tubing in the apparatus is capillary, of 6–7 mm. external diameter, and of 1 mm. bore, except the tube from A, which is of 2 mm. bore. Cylinder A, of 35 c.c. capacity, serves to hold water which is

[1] The apparatus is furnished by E. Machlett and Son, 143 E. 23 St., New York City ($12) and by Robert Goetze, Leipzig (Mk. 25).

used to displace air from D, or to receive solution forced back from D by nitric oxide. The 10 c.c. burette B holds the solution of amino
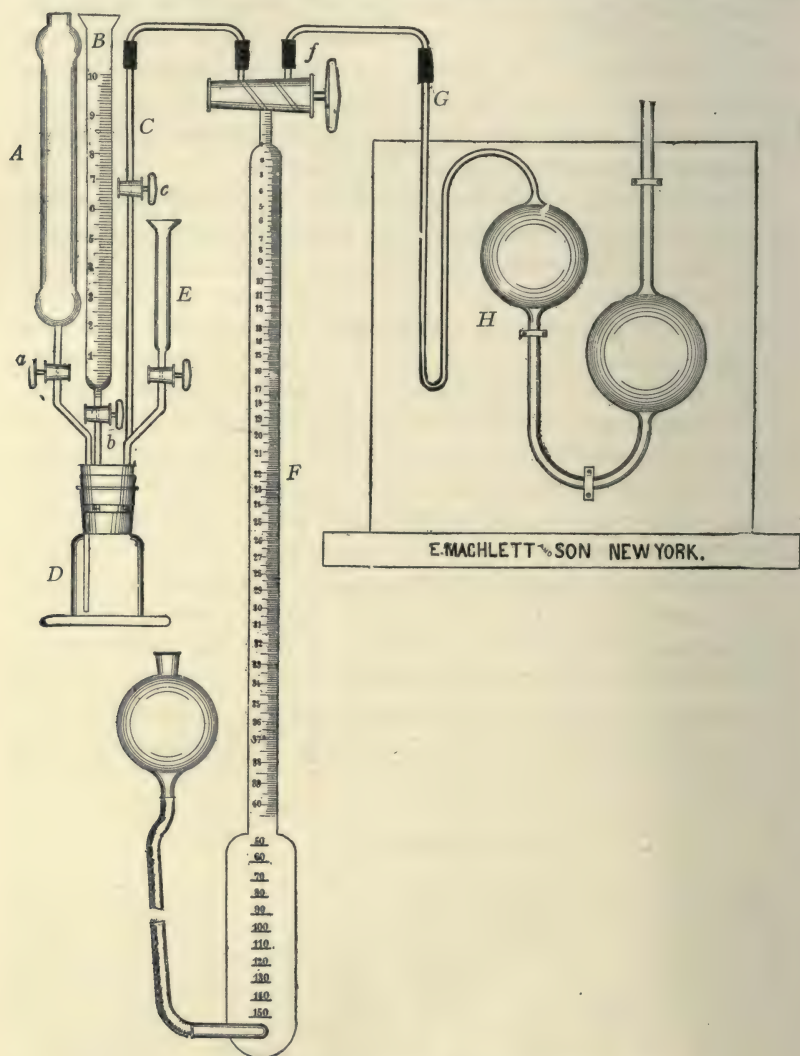
FIG. 12.—Van Slyke's Amino Nitrager Apparatus.

substance for analysis. Tube C serves as an outlet for gases, and connects D with the gas burette while the nitrogen is being evolved.

The lower end of C is exactly flush with the bottom of the stopper. The small cylinder E, of 2 c.c. capacity, holds amyl alcohol for use in analysis of viscous solutions, such as those containing albumoses, or proteins. The addition of an occasional drop of amyl alcohol prevents foaming of these solutions during the evolution of nitrogen. The gas burette F is divided into tenths of a c.c. for 40 c.c. Below the 40 c.c. mark it broadens into a bulb, which is graduated only into 10 c.c. divisions. The bulb provides a volume capable of holding the mixture of nitrogen and nitric oxide first liberated, while the finely divided portion of the burette measures the pure nitrogen after the oxide has been absorbed. The water in the gas burette dissolves some of the nitric oxide, which keeps the burette clean by reducing the occasional drops of permanganate carried back with gas from the absorption pipette. Capillary rubber tubing with walls 3 or 4 mm. thick is used to connect C and G with the gas burette. The absorbent solution in the Hempel pipette is the alkaline permanganate already described.

The Determination.

The process may be divided into three stages: (1) Displacement of the air in the apparatus by an atmosphere of pure nitric oxide; (2) Decomposition of the amino substance; (3) Absorption of nitric oxide and measurement of the pure nitrogen. The entire determination usually requires about 10 minutes.

Displacement of Air by Nitric Oxide.—The solution of amino substance, containing preferably not over 20 mg. of amino nitrogen, is placed in B, and 5 c.c. of water in A. Into D one then pours 28 c.c. of the solution of sodium nitrite (30 gm. to 100 c.c. of water) followed by 7 c.c. of glacial acetic acid. Rapid evolution of nitric oxide begins at once. The cock c being open, the stopper is now placed in the neck of D and fastened firmly with the wire. The small volume of air in D is driven out by letting in the water from A until the bottle is completely filled and liquid rises in C. In order to remove also the air dissolved in the nitrous acid solution, c is closed, a left open, and D is shaken, the tops of A, B, and C being held by the left hand. The shaking causes quick evolution of nitric oxide, which gathers in the top of D and forces 10–15 c.c. of solution back into A. Cock c is now reopened, and the nitric oxide, together with the air which it has swept out of the solution, is forced out of D by liquid from A. In order to

assure complete removal of all traces of air, c is closed and the process
once repeated. Then, by again closing c and shaking D, one generates
a gas space of about 20 c.c. in D, in order to make room for the amino
solution from B. G and H being completely filled with permanganate
solution, and F with the 1 % sulphuric up to the top of the rubber
connecting tube, C and F are joined, cock b being opened and a
closed. The above manipulations require about 2 minutes.

Decomposition of the Amino Substance.—C and F being connected,
the amino solution from B is run into D, and mixed with the nitrous
acid solution. Rapid evolution of nitrogen, mixed with nitric oxide,
begins at once. After the reaction has run 5 minutes, in the case of
the α-amino-acids, or longer, as required for most other amino
derviatives, the evolution of nitrogen is completed by thoroughly
shaking D.

If proteïns, albumoses, or other substances producing viscous solu-
tions are present in the amino solution, a drop of amyl alcohol is
occasionally added (from E, Fig. 12) to prevent foaming during
the rapid evolution of nitrogen. When, as in digestion experiments,
the determination is performed upon proteins or their partially hydro-
lysed products, the reaction is run from only 5 minutes, the solution
being stirred by shaking several times a minute. Under these condi-
tions there appears to be no danger of decomposition, other than deamin-
·ation, of the complex substances. The deaminised products, from
the proteins and their primary hydrolytic products, are insoluble.
Consequently precipitates result from the action of nitrous acid on
solutions of proteins undigested or in the earlier stages of digestion.
The precipitates do not interfere at all with the determinations. In
case ammonia, which does not react as rapidly as primary amino
groups, is present, about 15 % of it is converted into free nitrogen
during the 5-minute reaction at 20°.

Absorption of Nitric Oxide and Measurement of Nitrogen.—The
reaction being completed, all the gas is driven from D and C into
F by opening a and letting liquid from A into D. By raising the
levelling bulb the gas is driven from F into H, care being taken that
none is left in the connecting capillaries of G and the pipette. The
nitric oxide is absorbed by shaking the gases with the permanganate
solution. The pure nitrogen is run back into F, the permanganate
filling G as far as f. The surface of the water in the levelling bulb
being brought even with the meniscus, the volume of gas in F is

measured. The absorption usually occupies about a minute, but varies somewhat with the volume of the nitrogen, the freshness of the permanganate, and the thoroughness of the shaking. It is advisable, until one has a little experience, to test the completeness of the absorption by repeating it, and noting whether the volume of gas is diminished. The room temperature beside the apparatus and the atmospheric pressure are taken, and the weight of nitrogen calculated from the usual tables for nitrogen gas measured over water. As the reaction gives double the amount of nitrogen present in the amino groups, the results are to be divided by 2. Consequently, each mgrm. of amino nitrogen generates, according to pressure and temperature, 1.7–1.9 c.c. of nitrogen gas, which enables one to obtain very accurate results with relatively small amounts of material.

In the method as above described the only source of error, reagents being pure, is the 0.2 c.c. of air which the 10 c.c. of amino solution can dissolve at atmospheric pressure. As the oxygen combines with the NO to form NO_2, which is absorbed by the permanganate, only the 0.16 c.c. of nitrogen is added to the gas measured. This correction is also indicated by blank experiments. Consequently when the amino solution is saturated with air, 0.16 c.c. is deducted from the nitrogen volume. The correction, which is equivalent to only 0.09 mg. of amino nitrogen, can be avoided by using, in preparation of the amino solution, water which has been freed from air by boiling, or by shaking for a few seconds in an evacuated flask.

Time Required for Different Classes of Amino Derivatives to react Quantitatively.—Amino groups in the α-position to carboxyl, as in the natural amino-acids, react quantitatively in 5 minutes at 20° The group in *lysine* requires 30 minutes to react completely, lyisne being the only natural amino-acid which requires more than 5 minutes. *Ammonia and methylamine* require 1.5–2 hours to react quantitatively. *Urea* requires 8 hours. In 1 hour it gives off 50% of its nitrogen, and the reaction rate follows the monomolecular equation. Amino groups in *purines* and *pyrimidines* require 2 to 5 hours at 20°.

In case, for any reason, there is doubt concerning the completeness of the reaction, C and F are left connected, a being open, while the nitric oxide is absorbed and the nitrogen measured. The gas which has meantime collected in the top of D, together with that which can be freed from the solution in D by shaking, is run over into F, freed from nitric oxide, and the nitrogen is again measured. If there is no

increase in the nitrogen volume, the reaction was complete at the first measurement.

The following table indicates in a general way the content of different proteïns in amino-acids. The figures are all too low, a fairer idea would be obtained if the figures were all doubled, though in the case of tyrosine and cystin the figures stated are substantially correct.

AMINO-ACIDS IN DIFFERENT PROTEINS.

Proteïns	Ammonia	Lysine	Arginine	Valine	Oxyproline	Proline	Leucine	Glutamic acid	Aspartic acid	Tryptophane	Histidine	Cystin	Serine	Tyrosine	Phenylalanine	Alanine	Glycocoll
Serum albumin	+	+	1	..	+	1	20	8	3	+	....	2.3	1	2.	3.	3.	o
Egg albumin	+	2	2	..	+	2	8	8	2	+	+	.2	+	1.	4.	8.	o
Lac-albumin	+	+	+	+	1	4	19	10	1	+	+	+	+	1.	2.	53.	o
Serum globulin	+	..	+	+	..	3	18	8	2	+	....	1.	..	2.	54.	2.	3.5
Cotton-seed edestin	+	1	11	+	2	2	20	6	4	+	1.	+	1	2.	2.	4.	4.
Sun-flower edestin	+	..	..	+	..	3	13	13	3	+	....	....	1	2.	4.	4.	2.5
Globulin from toga-bean	20	3	5	1	..	4	9	19	4	+	1.4	....	..	2.	4.	....	1.
Legumin	..	5	5	1	..	6	9	16	4	...	1.	....	..	3.	2.	3.	1.
Gliadin	5	o	3	+	..	2	6	36	1	1.	2.	+	+	2.	2.	53.	1.
Zein	3	o	1	+	..	6	18	18	1	o	+	....	+	3.	5.	2.	o
Hordein	5	o	2	..	..	9	6	33	+	+	+	....	+	1.	3.	1.	+
Glutin	4	2	5	+	..	4	6	23	+	+	2.	+	+	4.	2.	5.	+
Leucosin	1	3	6	+	..	3	11	6	3	+	3.	....	..	3.	4.	4.	+
Avenin	..	..	..	2	..	5	15	18	4	....	....	....	..	1.	3.	2.	1.
Fibrin	+	4	3	1	..	4	15	10	2	+	+	1.	1	3.	3.	4.	3.
Casein, cow	..	6	5	1	+	3	10	11	1	1.5	3.	+	+	5.	3.	1.	o
Thymus, histon	..	7	15	..	..	1	12	+	..	....	2.	....	..	5.	2.	3.	+
Globin, hemoglobin	..	4	5	..	1	2	29	2	4	+	11.	.3	1	2.	4.	4.	o
Protamine, salmon	o	o	87	2	o	7	..	o	o	o	o	o	5	o	o	o	o
Strurin	..	12	60	o	o	o	..	..	..	o	13.	o	o	o	o	+	o
Scombin	o	o	89	o	o	4	..	o	o	o	o	o	o	o	o	7.	o
Silk fibroin	o	+	1	o	..	+	..	o	+	...	+	...	2	10.	1.	2.1	3.6
Elastin	..	..	+	1	..	2	21	+	+	....	....	....	..	+	4.	6.	2.6
Spongin	+	..	+	..	..	7	..	18	5	....	....	7.	+	...	o	o	14.
Keratin	+	..	3	5	..	4	18	3	3	....	....	...	..	5.	3.	1.	+
Wool keratin	..	..	..	..	..	4	12	12	2	....	....	8.	+	...	o	4.	1.
Gelatin	..	3	8	1	3	5	2	1	+	o	+	2.	2.	o	.5	1.	16.

BETAÏNES.

On page 212, it was stated that glycocoll was the starting-point of two distinct series of bases. One series, having the characters of amino-acids, is represented by leucine, tyrosine, and asparagine, and has already been considered. The bases of the second series may be termed methyl-bases. The following are the most important bases of this class:

It will be seen that glycocoll, sarcosine, and betaïne may be regarded as internal anhydrides, thus differing by the elements of water from the remaining members of the group, which have the constitution of hydroxides of ammonium-bases.

		Base	Salt
Glycocoll (page 207),	$C_2H_5O_2N$	$N \left\{ \begin{array}{l} H_3 \\ CH_2.CO \\ O \text{ ——} \end{array} \right\}$	$N \left\{ \begin{array}{l} H_3 \\ CH_2.COOH \\ Cl \end{array} \right.$
Sarcosine (page 272), (Methyl-glycocoll).	$C_3H_7O_2N$	$N \left\{ \begin{array}{l} (CH_3)H_2 \\ CH_2.CO \\ O \text{ ——} \end{array} \right\}$	$N \left\{ \begin{array}{l} (CH_3)H_2 \\ CH_2.COOH \\ Cl \end{array} \right.$
Betaïne (page 273), (Trimethyl-glycocoll.) (Dimethyl-sarcosine.)	$C_5H_{11}O_2N$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH_2.CO \\ O \text{ ——} \end{array} \right\}$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH_2.COOH \\ Cl \end{array} \right.$
Neurine (page 274),	$C_5H_{13}ON$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH:CH_2 \\ OH \end{array} \right.$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH:CH_2 \\ Cl \end{array} \right.$
Choline (page 276),	$C_5H_{15}O_2N$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH_2.CH_2(OH) \\ OH \end{array} \right.$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH_2.CH_2(OH) \\ Cl \end{array} \right.$
Muscarine (page 284), (Hydroxy-choline.)	$C_5H_{15}O_3N$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH_2.CH(OH)_2 \\ OH \end{array} \right.$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH_2.CH(OH)_2 \\ Cl \end{array} \right.$
Isomuscarine (page 286),	$C_5H_{15}O_3N$	$N \left\{ \begin{array}{l} (CH_3)_3 \\ CH(OH).CH_2(OH) \\ OH \end{array} \right.$	. .

If muscarine has an aldehydic constitution, its toxic character is probably due to presence of the aldehydrol group $-CH_2.CH(OH)_2$, or of the group $-CH_2.COH$; since the groups $-CH_2.CH_2.OH$ and $-CH_2.COOH$, in combination with trimethylamine, have no direct poisonous action. In the case of neurine, on the contrary, it is probable that the toxic action may be connected with the double linking in the vinyl-group $-CH:CH_2$. Hence it might be expected that a corresponding trimethylamine derivative with a triple linking would have a similar or even stronger toxic action. This inference has been found correct, acetenyl-trimethylammonium hydroxide, $(CH_3)_3.(OH).C:CH$, being a more powerful poison even than neurine. On the other hand, allyl-trimethylammonium hydroxide, which has the constitution of a higher homologue of neurine, is a comparatively non-poisonous substance.

It is interesting to observe that while muscarine is intensely poisonous, the isomeric base isomuscarine is relatively innocuous. Hence the toxic character of the former substance appears to depend upon the existence of two loosely-combined hydroxyl-groups attached to the same carbonatom.

Sarcosine.   Methyl-glycocoll.

$$CH_2N \underset{H}{\overset{CH_3}{\diagup}}$$

C₃H₇O₂N; i.e.,

$$CH_2N<^{CH_3}_{H}$$
$$|$$
$$COOH$$
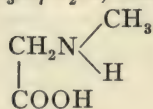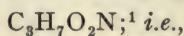
Sarcosine is prepared by boiling creatine with an aqueous solution of 10 times its weight of barium hydroxide until all odour of ammonia has disappeared. The creatine is decomposed into sarcosine and urea, the latter product being further split up into ammonia and carbonic acid. The excess of barium hydroxide is removed by a current of carbon dioxide, the liquid boiled, filtered, and evaporated to a syrup, from which the sarcosine is deposited in foliated crystals on standing. Sarcosine also results from the action of acids or alkalies on caffeine and theobromine (Vol. VI); and W. Paulmann (*Arch. Pharm.*, **232,** 601) recommends the hydrolysis of caffeine as the best method for the preparation of sarcosine, the yield being 60 % of the theoretical.

Sarcosine has been obtained synthetically by digesting ethyl chloracetate under pressure, at 125°, with an excess of a concentrated s o l u t i o n of methylamine: $CH_2Cl.COO(C_2H_5)+NH_2Me+H_2O=$ $NHMe.CH_2.COOH+C_2H_5.OH+HCl$. The yield by this method is very low.

Sarcosine may be purified by conversion into the sulphate, the aqueous solution of which is then decomposed by pure barium carbonate.

Sarcosine crystallises in colourless, transparent, rhombic prisms, having a sharp, sweetish, somewhat metallic taste. Sarcosine is unchanged at 100°, but at a higher temperature melts and volatilises without leaving any residue.

Sarcosine is readily soluble in water, sparingly soluble in alcohol, and insoluble in ether. It has no action on litmus, but combines with acids to form soluble crystallisable salts. B_2,H_2SO_4+2 H_2O forms colourless, quadrangular crystals, very readily soluble in water. B_2,H_2PtCl_6+2 H_2O is soluble in water, and crystallises in large, pale yellow, flattened octahedra. Sarcosine also reacts with bases. The *cupric salt*, $Cu(C_3H_6NO_2)_2+2$ H_2O forms deep blue crystals.

When heated with soda-lime, sarcosine evolves methylamine.

---

[1] Sarcosine is isomeric with alanine, lactamide, and urethane. It is distinguished from these substances by its insolubility in alcohol and ether, in addition, of course, to various chemical reactions.

Benzoyl-sarcosine (methyl-hippuric acid), NMeBz.CH.COOH, has been prepared, but owing to its extreme solubility has not been obtained in crystals.

In all its chemical relationships sarcosine presents a close resemblance to glycocoll.

<div align="center">

Betaïne. Dimethyl-sarcosine. Oxycholine.

</div>

$$(H_3C)_3N\diagdown\!\!\!\!\!\diagup\substack{O \\ \\ \\ C \\ H\;\;H}\!\!\!\!\diagdown\!\!\!\!\!\diagup CO$$
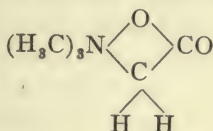
Betaïne is usually regarded as having the constitution of an internal anhydride.

Betaïne occurs naturally in the juice of beetroot (*Beta vulgaris*). The unripe root contains 0.25, but the ripe root only 0.10%. The betaïne is not present in the root as such, but in a form from which it may be liberated by treatment with hydrochloric acid or barium hydroxide. Hence the compound is probably allied to the lecithins (page 280). Betaïne is also present in beetroot molasses, in the branches and leaves of *Lycium barbarum* (whence its name lycine), in mangoldwurzel, in cotton-seed, etc., and is a product of the decomposition of proteins.

For the preparation of betaïne, beetroot juice or molasses should be diluted with water and treated with lead acetate in slight excess. The precipitate, which contains the betaïne, is decomposed by dilute sulphuric acid, and the solution precipitated by phosphotungstic acid. The resultant precipitate gives free betaïne when treated with milk of lime.

Another plan is to boil the diluted molasses or beet-juice with barium hydroxide for twelve hours, filter, pass carbon dioxide, evaporate the filtered liquid to a syrup, and exhaust with alcohol. The extract is treated with an alcoholic solution of zinc chloride, the precipitate separated, recrystallised from water, and decomposed by barium hydroxide. The solution is exactly decomposed by dilute sulphuric acid, and the filtered liquid evaporated till the betaïne hydrochloride crystallises out.[1]

---

[1] Natural betaïne is the type of a series of similar bases obtainable synthetically. These may be prepared with but slight admixture of secondary products by the action of alkyl iodides on zinc salts of the amino-acids in presence of zinc oxide (E. Duvillier, *Compt. rend.* **110**, 640, and *J. Chem. Soc.*, **58**, 747).

Betaïne has also been obtained by the oxidation of choline, by the action of chloracetic acid on trimethylamine, and by treating a potassium hydroxide solution of glycocoll with methyl iodide and methyl alcohol.

Betaïne crystallises from alcohol in large deliquescent crystals containing 1 H_2O which is lost at 100°, or by exposure over strong sulphuric acid. It is precipitated in scales on adding ether to its alcoholic solution. Betaïne is optically inactive, has a sweet taste, is not poisonous, and is neutral to litmus. When heated, it decomposes with evolution of trimethylamine, and gives an odour of burnt sugar.

Betaïne is not affected by chromic or hydriodic acid; but on boiling or fusion with alkali hydroxide it yields trimethylamine.

The *salts* of betaïne are regarded as direct compounds of $C_5H_{11}O_2N$ with acids. Thus, the compound $C_5H_{12}O_2NCl$ is betaïne hydrochloride, $C_5H_{11}O_2NHCl$. It forms large, stable, monoclinic tables, melting with intumescence at 228° and very readily soluble in water, but nearly insoluble in absolute alcohol (distinction from choline). $(C_5H_{12}O_2N)_2PtCl_6$ forms large, yellow, efflorescent crystals, deposited from water in hexagonal plates, and from dilute alcohol in hydrated octahedra. The water of crystallisation is variously stated at 2, 3, and 4 molecules. The gold salt crystallises in thin needles or plates resembling cholesterol, soluble in water and melting at 209°. $B,ZnCl_2$ is crystallisable, soluble in water, but insoluble in strong alcohol.

With Mayer's reagent, a solution of betaïne hydrochloride yields a whitish-yellow precipitate, soluble in excess; but if the sides of the glass containing the precipitate be rubbed with a glass rod, yellow needles are deposited.

A solution of iodised potassium iodide precipitates betaïne as a periodide in brown crystals.

Betaïne derived from the plant world will pass through the animal body unchanged, but it has never otherwise been found in the animal body nor is there evidence that it is ever originated there, either from choline or otherwise.

Neurine. Vinyl-trimethylammonium Hydroxide.

$$C_5H_{13}ON; \; i.e.,$$
$$HO.N \Big\langle\!\!\!{}^{(CH_3)_3}_{CH:CH_2}$$
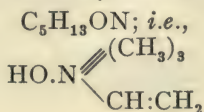
This base, discovered by A. W. Hofmann, differs from choline (page 276) by the elements of water. It occurs with choline in various animal substances and the products of their decomposition. It is now believed that neurine occurs in nature only as the result of the bacterial decomposition of choline. Even so, particular circumstances must be essential to its formation, since it is not always to be found in decomposed tissue of the central nervous system, in which choline is largely set free. Neurine is not formed in the ordinary hydrolysis of the white matter of the brain by barium hydroxide solution.

Free neurine is only known in aqueous solution. It has a strong alkaline reaction, and absorbs carbon dioxide from the air.

Choline can be converted into neurine by heating it to 140° with fuming hydriodic acid, and eliminating the iodine from the product by moist silver oxide. The reverse reaction has also been effected by heating neurine chloride with hydriodic acid, and then heating the product with silver nitrate in aqueous solution.

Neurine and choline present very close resemblances, and hence the few distinctions between them are important. Thus neurine chloride gives an abundant precipitate with tannin, while the choline salt is not affected. On the other hand, choline chloride is precipitated by phosphotungstic acid, which with neurine gives no reaction. Choline platinichloride forms large, soluble, red, tabular, monoclinic crystals, arranged like steps. They melt in a capillary tube at 232 to 233°, but generally at 240 to 241° with much frothing. The platinum salt of neurine contains $(C_5H_{12}NCl)_2,PtCl_4$, and crystallises in small, individual, orange-red, regular octahedra, which melt at 211 to 213°, and dissolve with difficulty in hot water. The crystals soon turn opaque, and on retreatment with water leave an insoluble residue, while the platinum salt of choline is found in the solution.

F. Marino-Zuco (*Gazzetta*, **13,** 431) has pointed out that neurine chloride is not decomposed by sodium hydrogen carbonate, in which it differs from the hydrochlorides of most of the poisonous vegetable alkaloids. Hence if the mixed alkaloids and ptomaïnes, simultaneously extracted by Stas' process, be dissolved in hydrochloric acid, and the solution treated with sodium hydrogen carbonate, the vegetable alkaloids can be extracted by agitation with ether, chloroform, amyl alcohol, etc., while the neurine and other soluble ptomaïnes remain in the aqueous liquid.

Neurine is extremely poisonous, the symptoms produced resembling

those due to poisoning by muscarine.   Administered to a frog subcutaneously, it soon produces paralysis of the extremities, which is followed by stoppage of the respiration, and finally of the heart (in diastole).   In rabbits, neurine occasions profuse nasal secretion, salivation, and paralysis.   Neurine produces contraction of the pupil, both when injected and when applied locally.   Atropine has been found to be an efficient antidote, and even produces temporary immunity to poisoning by neurine.   Hydroxy-trimethyl-ammonium compounds are stated by V. Cervello to act similarly.

Choline.   Trimethylhydroxyethylammonium hydroxide.

$$C_5H_{15}O_2N; \; i.e.,$$
$$HO.N \underset{CH_2.CH_2.OH}{\overset{(CH_3)_3}{<}}$$
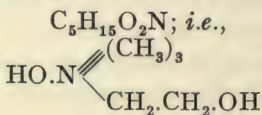
Choline has been prepared synthetically by treating ethylene oxide with a concentrated solution of trimethylamine.

Choline is a decomposition-product of some lecithins, but also exists ready-formed in the tissues of living animals and plants, and is one of the first and most constant products of the putrefactive decomposition of nervous tissue.   It was first isolated from bile (whence the names choline and bilineurine), has been found in herring brine, and exists also in the brain and in yolk of egg, in the conjugate form of a lecithin.

It is the constant associate of neuridine during the earlier stages of putrefaction, being afterward replaced by trimethylamine, which is no doubt produced by the decomposition of choline itself—$C_5H_{15}NO = C_2H_6O_2 + C_3H_9N$.   Choline may be isolated from putrefying matters by adding picric acid to the mother-liquor from which neuridine has been separated.

Besides occurring naturally in the animal kingdom, choline exists in a large number of plants and plant products.   It has been shown to be identical with the base sincaline, obtained by the decomposition of sinapine occurring in white mustard seeds.   It has also been found in many fungi, in germinated pumpkin sprouts, in the seeds of cotton (see page 278), *Vicia sativa* (vetch), *Trigonella Fœnum græcum* (fenugreek), ergot of rye, areca nuts, the fly agaric, ipecacuanha and hops; and was extracted by P. Griess from beer, which, according to J. Kjeldahl, also contains a choline derivative (lecithin).[1]

[1] Kjeldahl found that the proportion of choline in beer was the same as that in the wort prior to fermentation.   To extract it, he evaporates the beer or wort to one-half, and treats it with excess of milk of lime and one or two volumes of alcohol.   The filtrate is acidified

Choline may be prepared from yolk of egg or lecithin by exhausting the substance with ether and afterwards with warm alcohol. These are distilled from the extract, the residue boiled for an hour with barium hydroxide, the excess of barium precipitated by carbon dioxide, and the filtrate evaporated. The residue is exhausted with absolute alcohol, and the solution precipitated by platinic chloride. The platinichloride is dissolved in water, and decomposed by hydrogen sulphide. The solution of choline chloride is concentrated, and treated with silver oxide, when a strongly alkaline solution of free choline is obtained, and on evaporation the base remains as a syrupy liquid.

Free choline is a deliquescent substance very difficult to crystallise. It usually forms a syrup. It is a powerful base, having an alkaline reaction, and absorbing carbon dioxide from the air.

Choline, having the constitution of a tetralkylammonium hydroxide (compare Vol. VI), forms salts by the replacement of the OH group by Cl, I, SO_4, etc. Thus the *chloride* has the formula (C_2H_4OH)-$(CH_3)_3N.Cl$, and crystallises from absolute alcohol in fine deliquescent needles, readily soluble in alcohol and water (distinction from betaïne). The *platinum salt* has the composition $[(C_2H_4OH)(CH_3)_3NCl]_2,PtCl_4$, and crystallises from hot water in fine reddish-yellow plates or prisms, insoluble in absolute alcohol (compare page 275). The *gold salt* (Au = 44.5 %) is deposited from a hot saturated aqueous solution in long yellow prisms, which melt at 244 to 245°, and are soluble with difficulty in cold water, but dissolved by hot water or alcohol. *Choline sulphate* is amorphous, and almost insoluble in absolute alcohol, but very soluble in water. The *carbonate* is amorphous, very deliquescent, alkaline in reaction, and soluble in alcohol.

When choline is treated with hydriodic acid, both the hydroxyl-groups are replaced with formation of the substance $(C_2H_4I)(CH_3)_3NI$; and when this is treated with moist oxide of silver and water (compare Vol. VI,) it yields the base neurine, $(C_2H_3)(CH_3)_3N.OH$. On oxidation, choline is converted into betaïne, $(CH_2.COOH)(CH_3)_3N.OH$.

Choline is precipitated as an insoluble salt by potassium iodide and iodine (or by potassium tri-iodide). There is no known method for the quantitative estimation of choline in animal material, though Stanek

with sulphuric acid, evaporated on the water-bath with excess of barium carbonate till the alcohol is volatilised, and a large excess of iodised potassium iodide added. Needles of choline polyiodide, exhibiting a beetle-green reflection, are gradually deposited. These are exactly decomposed by sulphurous acid, the solution shaken with silver chloride (to convert the iodide into chloride), and the filtered liquid treated with platinic chloride to obtain the choline as a platinum salt.

has worked out a method for the estimation in molasses (*Zeitschr. Physiol. Chem.*, **47**, 147, 54). Probably it could be approximated by initial precipitation with mercuric chloride, collection and washing of the precipitate, precipitation of the mercury with hydrogen sulphide and final precipitation in the filtrate with platinic chloride.

Owing to the poisonous character of choline[1], W. Maxwell (*Amer. Chem. J.*, 1891, **13**, 469) has endeavoured to find out whether choline and betaïne are present in the cotton seed from which various cattle foods are prepared. Betaïne is generally believed to be non-poisonous, but is usually found together with choline.

About 5 pounds of finely ground cotton-seed cake was extracted with 70% alcohol, the extract distilled, and the residue taken up in water. On adding lead acetate to this solution, a precipitate was thrown down which was separated, and the filtrate evaporated to a syrup after the excess of lead had been removed. The alkaloidal substances were then taken up from this syrup in a mixture of 70% alcohol and 1% hydrochloric acid. This extract was treated with an alcoholic solution of mercuric chloride, when immediately an almost pure white double salt of the nitrogenous bases began to separate out. After standing for 10 days the crystals were separated from the liquid, from which more crystals were deposited after some weeks. After recrystallisation from water the salt was decomposed by means of hydrogen sulphide. The filtrate, containing the chlorides of the bases, was slowly evaporated, and then placed in a desiccator over sulphuric acid until crystallisation of the salts was complete. The crystals, which were free from colour and well-developed, after drying, were saturated with absolute alcohol, in which the choline salt dissolved along with a small proportion of the betaïne salt. 7.248 grm. of the crystals were treated with alcohol, and the extract evaporated to dryness and reextracted three times to obtain the choline salt free from betaïne. There were thus obtained 1.08 grm. of choline chloride, and 6.168 grm. of the corresponding betaïne salt. Choline and betaïne appeared to be present in the sample of cattle-food used by Maxwell in the relative proportions of 17.5% choline to 82.5% betaïne.

The alcoholic solution of the choline salt was treated with platinic chloride, and choline platinichloride obtained. From this an aqueous solution of choline chloride was obtained by treatment with hydrogen

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[1] Brieger found a dose of 0.005 grm. of choline chloride requisite to cause death to a rabbit, while one-tenth of that amount of neurine chloride proved fatal.

sulphide, and the separation of the resulting platinic sulphide by filtration. This aqueous solution gave the following reactions:

With Phospho-tungstic acid,   white precipitate.
      Phospho-molybdic acid,   yellow precipitate.
      Bismuth-potassium iodide,   red precipitate.
      Cadmium-potassium iodide, grey precipitate.
      Iodine,   brown precipitate.
      Platinic chloride,   yellow precipitate soluble in water.

An aqueous solution of the betaïne hydrochloride was treated with phospho-tungstic acid, the precipitate treated with milk of lime, and the resulting lime salt filtered off. The residue was evaporated and extracted with strong alcohol, from which free betaïne crystallised out.

Schulze and Frankfurt (*Ber.*, 1893, **26**, 2151–2155) have described the following process for the isolation of betaïne and choline existing in malt-culms and wheat-germs. The material is extracted with water, lead acetate added so long as a precipitate is produced, the solution acidified with sulphuric acid, and filtered. Phospho-tungstic acid is then added, and the resulting precipitate washed and treated in the cold with milk of lime. The filtrate from the insoluble calcium compounds is treated with carbon dioxide to remove the excess of lime, filtered, neutralised with hydrochloric acid, evaporated to a syrup, and the latter extracted with hot 90–95 % alcohol. Alcoholic mercuric chloride solution is added to the extract, which is then allowed to stand for several days, when the separated mercury double salt is removed and crystallised from water. The difficultly soluble portion of the salt contains the betaïne, while the easily soluble part consists of the choline compound. These can be separated by repeated fractional crystallisation from water, or by decomposing with hydrogen sulphide and treating the hydrochlorides so obtained with cold absolute alcohol. The choline salt dissolves while the betaïne compound remains behind.

Three kilos. of wheat embryos yielded 5 to 6 grm. of betaïne hydrochloride, the yield of the choline salt being considerably less. Malt rootlets yielded a somewhat less amount of the betaïne salt, but a rather larger quantity of the choline compound.

E. Jahns (*Ber.*, 1893, **26**, 1493; *Pharm. J.*, [iii], **24**, 245) isolated choline and betaïne from worm-seed (*Artemisia Gallica*, Wild.) by the

following process: The seed was extracted with hot water, and the liquor precipitated with lead acetate and soda. From the filtrate the excess of lead was thrown down by sodium phosphate, the filtrate evaporated to a small bulk, acidified with sulphuric acid and shaken with chloroform, which removed a bitter resinous substance and a little santonin. From the aqueous layer, mixed with a large proportion of sulphuric acid, the bases were precipitated by potassio-bismuth iodide, the precipitate washed with dilute sulphuric acid, and the choline and betaïne liberated by digesting the precipitate with freshly precipitated silver carbonate and water. The bases were separated by treating their chlorides with absolute alcohol, in which, at the ordinary temperature, the choline salt dissolves freely, while that of betaïne is almost insoluble.

Choline exists preformed in the tissue of the adrenal body, and indeed in many other organs. It is present in the blood in degenerative diseases of the central nervous system. It is commonly regarded as antagonistic to epinephrine, but this is very doubtful. The presence of choline in the body is to be regarded as the results of the normal disintegration of nervous tissue, and the bile is probably the fluid of elimination. Choline has been reported in the urine in Addison's disease.

Lecithins or Lecithines.

Vauquelin was the first to observe that the brain contains a phosphorised fat, which was later obtained from yolk of egg and caviare in a crystallised state by Hoppe-Seyler, who showed that the same substance, or others closely related to it, occurred very frequently in growing cells, both in the animal and the vegetable kingdom. Thus he isolated lecithins from yeast and various fungi, from seeds, and found it in all the organs and fluids of the human body except the gastric juice, the pancreatic secretion, the urine, and the saliva.

When a simple lecithin is boiled with barium hydroxide water it is saponified with formation of choline, glycero-phosphoric acid, and one or more fatty acids, which may be stearic, palmitic, or oleic acids. Fat-splitting ferments also hydrolyse lecithins. Lippmann obtained betaïne instead of choline by the hydrolysis of beetroot-lecithin. Hence it appears that the lecithins are a group of closely-allied com-

pounds, related to each other in much the same manner as the ordinary fats and cholesterols. The constitution of distearyl-lecithin is shown by the following formula:

$$CH_2.O.C_{18}H_{35}O$$
$$|$$
$$CH.O.C_{18}H_{35}O$$
$$|$$
$$CH._2O$$
$$HO {>} PO$$
$$C_2H_4 {-} O$$
$$N {<} (CH_3)_3$$
$$OH$$
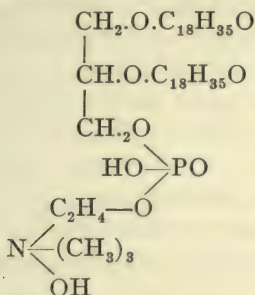
The simple lecithins are therefore mono-amino-phosphatides, formed by the substitution of 2 hydrogens of glycerol by fatty acids, the third hydrogen being replaced by phosphoric acid, to which is attached the base choline. The fatty acids included may be the same or different, and other bases than choline may be present. There are complex lecithins, composed possibly of several molecules united by the nitrogens of the base, or including other substances. The lecithins possess marked tendencies to the formation of adsorption compounds and are apparently essential components in protoplasm and especially in cellular membranes. Lecithin sugars have also been described.

For the preparation of lecithin, Diakonow directs that yolk of egg should be shaken up with ether as long as colouring matter is removed, when the residue is treated with water, filtered, and rapidly washed, and then digested with alcohol at 50–60°. The filtered liquid is quickly evaporated to the consistence of a syrup, the residue dissolved in a small quantity of absolute alcohol, and the filtered solution cooled by a freezing mixture. Lecithin separates gradually in nodular masses or (occasionally) crystalline tablets, while oleïn-lecithin remains in solution. Strecker prepares lecithin by extracting yolk of egg with ether-alcohol, distilling off the ether and adding alcohol to the residue as long as fats and other matters are precipitated. The filtered liquid is treated with an alcoholic solution of platinic chloride containing free hydrochloric acid, which produces a yellow flocculent precipitate of lecithin platinichloride, a compound which is soluble in ether, chloroform, or benzene, but insoluble in alcohol. This is purified by repeated solution in ether and precipitation with alcohol, and is then decomposed in

ethereal solution by hydrogen sulphide. On evaporation to dryness, lecithin hydrochloride is obtained as a waxy mass, which is taken up by ether-alcohol and shaken with oxide of silver. The resultant silver chloride is filtered off and dissolved silver separated from the filtrate by hydrogen sulphide. On evaporation, pure lecithin remains. Lippmann prepared lecithin from beetroot by a similar method. This method does not give a quantitative yield.

Properties.—Lecithin is a translucent, wax-like, imperfectly crystalline substance, which is very hygroscopic and swells up on treatment with water to form an opalescent liquid or emulsion,[1] which is precipitated or coagulated by various neutral salts. It is soluble in alcohol, ether, chloroform, carbon disulphide, carbon tetrachloride, petroleum ether, and benzene, but not in acetone.

Lecithin is optically active, rotating usually to the right.

Lecithin combines both with bases and acids,[2] but its compounds readily undergo decomposition, as also does lecithin itself. An alcoholic solution of free lecithin decomposes slowly in the cold, and more rapidly on heating, and from a similar solution of lecithin hydrochloride free fatty acids separate after a time. From an ethereal solution of lecithin platinichloride, choline platinichloride gradually separates on standing. If an ethereal solution of lecithin be shaken with dilute sulphuric acid, choline passes into the acid liquid, while the ether contains distearyl-glycero-phosphoric acid, $(C_{18}H_{35}O)_2:C_3H_5.PO_4H_2$, a substance which forms a crystalline potassium salt. A similar decomposition occurs in the first stage of putrefaction of animal substances containing lecithin. It forms with cadmium and tungsten complex salts that are quite insoluble in alcohol but soluble in pure carbon disulphide and ether. Lead acetate precipitates lecithins from aqueous solution of ammoniacal reaction, but not from alcoholic solution.

By boiling with barium hydroxide, the molecule of lecithin is split up differently, the first products being the barium salts of fatty acids, and the choline ester of glycero-phosphoric acid, but the latter com-

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[1] When lecithin is treated with a moderate quantity of water, the products exhibit under the microscope curious filaments, spherules, and other forms, closely resembling the so-called myeline forms observed by Virchow when nerve-fibres are exposed for a long time to the action of water.

Similar myeline forms are produced by "protagon," a highly complex substance extracted from ox-brains by means of alcohol. Protagon is stated to have a composition corresponding to the formula $C_{160}H_{308}N_5PO_{35}$, is crystallisable, and resembles lecithin in yielding choline, fatty acids, and glycerol-phosphoric acid as decomposition-products.

[2] As lecithin forms definite (though unstable) compounds with acids, it has the characters of a base, and would be more appropriately spelt lecithine. In practice, this is very rarely done.

pound readily undergoes further decomposition with formation of choline and barium glycero-phosphate.

Lecithin occurs widely distributed in the vegetable kingdom, and is found in the ethereal extracts of plants together with glycerides, wax-like products, cholesterol, etc.

For the isolation of lecithin from such extracts, Schulze and Likiernik (*Ber.*, 1891 **24**, 71) operate as follows: Finely powdered plant-seeds (vetch and lupin) are first extracted with ether, when only a portion of the lecithin goes into solution. The insoluble residue is then digested with 50% alcohol (in some cases a little alkali being added to neutralise the free acid contained in the seeds), whereby the bulk of the lecithin is dissolved in a fairly pure state.[1] In order to purify the product thus obtained, the solvent is distilled off at 40–50°, and the residue treated with cold ether. The lecithin dissolves, and by shaking the solution thus obtained with water the impurities are taken up by the latter. An emulsion, however, forms on shaking the mixture, and crystals of common salt must be added to clear the ethereal solution. This clear solution, when gently evaporated, leaves a residue of lecithin, which is further purified by dissolving it in absolute alcohol and again concentrating. The lecithin separates as a pale yellow product, possessing the characteristic properties of this body; but it could not be obtained in a crystalline form. When saponified by barium hydroxide solution, choline, glycerophosphoric acid, and fatty acids result, and were separated and identified.[2] Both solid fatty acids and oleic acid were found in the products of saponification, so that the lecithin from plants, like that from the yolk of eggs, appears to be a mixture of several lecithins.[3]

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[1] B. von Bitto (*Zeits. Physiol. Chem.*) has pointed out the great difficulty attending the complete extraction of lecithin by Schulze's process. After exhausting the substance with ether, he recommends that the residue should be boiled from 20 to 30 times with alcohol.

[2] **Glycerophosphoric acid,** $(OH)_2:C_3H_5.H_2PO_4$, may be obtained synthetically by the action of phosphoric anhydride or glacial phosphoric acid on glycerol. It is produced by boiling lecithin with barium hydroxide or sodium hydroxide; and occurs normally in urine (0.015 grm. per litre) and in animal tissues, etc., containing lecithin.

Glycerophosphoric acid has not been obtained pure, as it is decomposed on evaporation. In its most concentrated condition it forms a yellowish syrupy liquid of a sweet-acid taste. For the nature of ordinary glycerophosphoric acid see Power and Tutin, *Trans.*, 1905, **87**, 249; Tutin and Hann, *Trans.*, 1906, **89**, 1749. For other references see Vol. 2, p. 452. Glycerophosphoric acid is dibasic, and forms salts which are mostly soluble in water but insoluble in alcohol. It may be precipitated as the lead salt.

Calcium glycerophosphate, $CaC_3H_7PO_6$, forms a white crystalline powder, freely soluble in cold water but precipitated from its solution on boiling. It has been proposed as a readily assimilable form of phosphorus for medicinal use.

[3] W. Maxwell (*Amer. Chem. J.*, **13**, No. 6) has observed that the inorganic phosphorus present in mature seeds becomes reorganized under the action of the processes occurring during incipient growth, and appears in the young plantlet in the organic form as a constituent of lecithin. Maxwell has further observed that the lecithin present in the egg of a hen becomes reorganised during the process of incubation, and is found in the form of a mineral phosphate in the bone of the chicken.

The best general method for the isolation of lecithin is extraction with large amounts of alcohol-ether in the cold, and the precipitation of the filtered extract with acetone, which precipitates the lecithins. These may then be washed in acetone, redissolved in alcohol-ether that has been warmed and the precipitation with acetone repeated.

The best method of estimating lecithin quantitatively would be to take the purified substance and make a nitrogen and phosphorus determination. If as is usually the case, the lecithin was not pure but a mixture of several lipoids, the ratio found to exist between the N and P would vary widely from that demanded by the theory. It is also possible to split off the methyl groups, and thus calculate the lecithin, though here again there is no guarantee that only the lecithin is methyl-containing.

The presence of lecithin in the seeds of plants leads to an error in the estimation of the fat contained by extraction with ether, as the lecithin also goes into solution, though to a varying extent. The error is small in the case of seeds rich in fatty matter, but when the ether extract only amounts to about 2% and the per cent. of lecithin reaches 1.2 to 1.3, as in the seeds of the vetch and of the pea, the error is very considerable, and an estimation of the phosphorus in the residue should be made and the quantity of extract free from lecithin thus obtained. One part of $Mg_2P_2O_7$ represents 7.27 parts of lecithin.

Muscarine. Choline-aldehyde.

$$C_5H_{15}O_2N; \text{ } i.e.,$$

$$\begin{array}{c} (CH_3)_3 \\ \diagup\!\!\!\diagup \\ HO.N \\ \diagdown \\ CH_2.CHO \end{array}$$
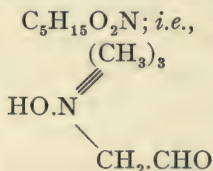
Muscarine is the poisonous principle of the toadstool known as the fly agaric (*Agaricus muscarius*), in which it occurs together with choline. Muscarine is also present in the fungus *Amanita pantherina*, and is a characteristic product, together with ethylene-diamine and gadinine, of the putrefaction of fish. It results from the oxidation of choline by strong nitric acid.

Muscarine forms thin laminæ or irregular crystals. It is very deliquescent, and is soluble in water and alcohol in all proportions, but is insoluble in ether and only with difficulty soluble in chloroform.

The aqueous solution of muscarine is strongly alkaline, absorbs

carbon dioxide from the air, and precipitates solutions of ferric and cupric salts.

The salts of muscarine are mostly very deliquescent, and neutral to litmus, except the carbonate, which is strongly alkaline. The salt produced by treating muscarine with hydrochloric acid is formed by the replacement of the hydroxyl-group, OH, by Cl, with elimination of water (compare page 271). The platinichloride $(C_5H_{14}ONCl)_2$-$PtCl_4 + 2H_2O$ forms well-defined octahedra, soluble in alcohol and difficultly soluble in water.

Muscarine salts yield amorphous precipitates with Mayer's reagent, the potassio-iodide of bismuth, with auric chloride, and with phospho-molybdic and phospho-tungstic acids. With Mayer's reagent muscarine salts yield a precipitate which is at first amorphous, but gradually becomes crystalline. Muscarine is not precipitated by tannin, picric acid, or iodised potassium iodide, and is not affected by boiling with dilute acids or alkalies.

According to G. Nothnagel (*Ber.*, 1893, **26**, 801) the artificial muscarine obtained from choline by oxidation with nitric acid agrees with the natural base in crystalline form, solubility, the composition of the platinum and gold salts, and to a large extent in its physiological action; but while artificial muscarine induces paralysis of the intermuscular nerve-terminations in the frog, and myosis in the pupil of the eye of birds, the natural base does not act in either of these ways.

Muscarine is tasteless but very poisonous, the action being narcotic and antagonistic to atropine. The heart action of a frog was arrested by 0.00003 grm. of muscarine, but recommenced its action on application of atropine. In its poisonous action muscarine resembles neurine. It produces a flow of saliva and tears, and paralyses and arrests the heart in diastole. Contraction of the pupil, diarrhœa, and emission of urine and semen are other notable symptoms. Administered in small doses, like pilocarpine it stimulates the secretion of the pancreatic gland.

Various other fungi besides the fly agaric produce marked symptoms of poisoning, but the active principles do not appear to be of alkaloidal nature; except, perhaps, in the case of *Agaricus ruber*, which is stated by T. L. Phipson to contain a colouring matter (ruberine) and the alkaloid *agarythrine* (*Chem. News*, **46**, 199).[1]

[1] The autumn fungus, *Agaricus phalloides*, which has not unfrequently been eaten in mistake for mushrooms, with fatal results, is said to owe its poisonous properties to a tox-albumin called phallin (see A. Wynter Blyth, *Poisons; their Effects and Detection*).

In man, from 3 to 5 mgrm. of muscarine injected hypodermically produce, in a few minutes, profuse salivation, rapid pulse, nausea, confusion of thought, giddiness, and myosis, but no vomiting or diarrhœa. Applied in small quantity to the eye, muscarine produces derangement of the accommodation but no change in the size of the pupils.   Larger quantities cause myosis.[1]   A minute amount of muscarine that could not be detected chemically may be detected by experimentation upon the isolated heart of the frog.   It is best to first test the particular heart with a known preparation of muscarine.

Isomuscarine is an isomer of muscarine obtained by synthetic means (see page 271).

UREA AND ITS ANALOGUES.

Urea is itself of pre-eminent interest and importance as the chief form in which the nitrogen of the protein ingested by man and other mammalia is eliminated from the system.   Urea is also the type of an extensive series of allied bodies and the nucleus of other compounds of natural origin and artificial synthetic formation.   The following is a list of the simpler and more typical members of the group:
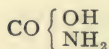
Carbamic Acid.[2]	Carbamide. (Urea.)	Imido–Urea. (Guanidine.)
$CO \begin{cases} OH \\ NH_2 \end{cases}$	$CO \begin{cases} NH_2 \\ NH_2 \end{cases}$	$C(NH) \begin{cases} NH_2 \\ NH_2 \end{cases}$
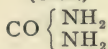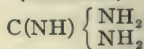
[1] For the detection of muscarine in cases of poisoning by the actual base, or by the fly-agaric, A. Wynter Blyth suggests that the matters should be treated with water acidified with hydrochloric acid, and the liquid concentrated to a syrup *in vacuo*.   The syrup should then be treated with water, and mercuric chloride added.   The excess of mercury is removed from the filtered liquid by hydrogen sulphide, and the filtrate evaporated to a syrup, which is then repeatedly extracted with alcohol, and the solution treated with platinic chloride.   The filtrate is freed from alcohol, treated with hydrogen sulphide and again filtered, the filtrate concentrated to a small volume, and platinic chloride again added, when the platinum salt of muscarine may be thrown down at once, or on further concentration.   Isolation of the muscarine by precipitation with Mayer's solution would probably be preferable to the foregoing scheme.
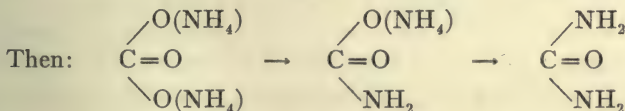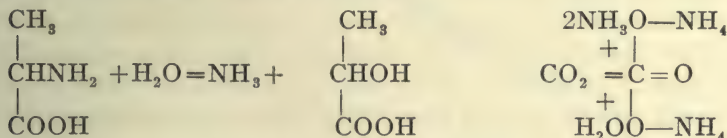
[2] Carbamic acid, $NH_2.COOH$, is not known in the free state.   The ammonium salt is formed by the direct union of carbon dioxide and dry ammonia gases, a second molecule of ammonia uniting with the nascent acid to form *ammonium carbamate*, $NH_2.COO(NH_4)$. This salt exists in commercial ammonium carbonate, and can be obtained by digesting that compound in strong ammonia for 30 to 40 hours (Divers).   Ammonium carbamate is extremely soluble in water, with which it gradually reacts to form ammonium carbonate, $(NH_4).O.CO.O(NH_4)$.   When heated to about 60°, at the ordinary pressure, ammonium carbamate is decomposed into ammonia and carbon dioxide, but when heated under pressure to 130 to 140° it yields urea, as it does also when submitted in aqueous solution to a rapidly alternating current of electricity.

Calcium carbamate is precipitated on adding lime and alcohol to a solution of ammonium carbamate cooled to 0°.   It forms a crystalline powder, soluble in water.   The solution rapidly decomposes with separation of calcium carbonate.

Salts of carbamic acid occur in serum, and are also stated to be formed by the oxidation of leucine, tyrosine, glycocoll, and albumin by potassium permanganate in alkaline solution.

Ethyl Carbamate.[1]
(Urethane.)

$$CO \begin{cases} O(C_2H_5) \\ NH_2 \end{cases}$$

Ethyl Urea.

$$CO \begin{cases} NH(C_2H_5) \\ NH_2 \end{cases}$$

Thio-Urea.

$$CS \begin{cases} NH_2 \\ NH_2 \end{cases}$$
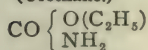
Phenyl-Urethane.[2]
(Euphorin.)

$$CO \begin{cases} O.(C_2H_5) \\ NH(C_6H_5) \end{cases}$$
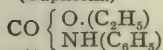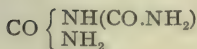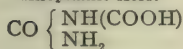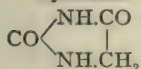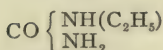
Diallyl-Urea.
(Sinapoline.)

$$CO \begin{cases} NH(C_3H_5) \\ NH(C_3H_5) \end{cases}$$
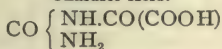
Allyl-Thiourea.
(Thiosinamine.)

$$CS \begin{cases} NH(C_3H_5) \\ NH_2 \end{cases}$$

Biuret.

$$CO \begin{cases} NH(CO.NH_2) \\ NH_2 \end{cases}$$

Acetyl-Urea.

$$CO \begin{cases} NH(CO.CH_3) \\ NH_2 \end{cases}$$
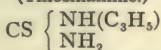
Phenetole-Urea.
(Dulcine. Sucrol.)

$$CO \begin{cases} NH.C_6H_4.(O.C_2H_5) \\ NH_2 \end{cases}$$

Allophanic Acid.

$$CO \begin{cases} NH(COOH) \\ NH_2 \end{cases}$$

Oxaluric Acid.

$$CO \begin{cases} NH.CO(COOH) \\ NH_2 \end{cases}$$

Alloxanic Acid.

$$CH \begin{cases} NH.CO.CO(COOH) \\ NH_2 \end{cases}$$

Hydantoin.

$$CO \begin{cases} NH.CO \\ | \\ NH.CH_2 \end{cases}$$

Parabanic Acid.

$$CO \begin{cases} NH.CO \\ | \\ NH.CO \end{cases}$$

Alloxan.

$$CO \begin{cases} NH.CO \\ NH.CO \end{cases} O$$
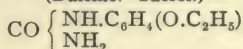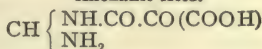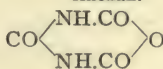
The larger portion of urea formed in the animal body is probably derived as follows. The amino-acids, products of the hydrolysis of protein, are deaminized, and the ammonia combines with the carbon dioxide of the circulating fluids to form ammonium carbonate. This is then converted into urea, through ammonium carbamate as intermediary stage. Thus for alanine:

$$\begin{array}{ccc}
CH_3 & CH_3 & 2NH_3O\text{---}NH_4 \\
| & | & + | \\
CHNH_2 + H_2O = NH_3 + & CHOH & CO_2 = C = O \\
| & | & + | \\
COOH & COOH & H_2OO\text{---}NH_4
\end{array}$$

$$\text{Then:} \quad C = O \begin{array}{c} {}^{O(NH_4)} \\ {}_{O(NH_4)} \end{array} \longrightarrow C = O \begin{array}{c} {}^{O(NH_4)} \\ {}_{NH_2} \end{array} \longrightarrow C = O \begin{array}{c} {}^{NH_2} \\ {}_{NH_2} \end{array}$$

one molecule of water being extruded in each stage.

Guanidine is described on page 304, and biuret on page 290. Thiosinamine is described under mustard oil (page 110), while oxaluric acid, parabanic acid, and alloxan are referred to under uric acid.

[1] *Ethyl carbamate* or *Urethane*, $NH_2.CO.O(C_2H_5)$, results from the action of aqueous ammonia on ethyl carbonate. It is also formed by the action of alcohol at 100° on urea or urea nitrate, and may be obtained by other methods. Traces of urethane exist in urine. Ethyl carbamate melts at about 50°, and distils at 182°. It is sparingly soluble in water, but readily soluble in alcohol and in ether. Treated in the cold with alcoholic potash, it yields crystals of potassium cyanate, KCNO. When heated with ammonia, urethane is converted into urea.

[2] *Phenyl-urethane*, $NH(C_6H_5).CO.O(C_2H_5)$, has been employed medicinally, as an antipyretic and antirheumatic, under the name of "euphorin." Acetyl and propionyl, derivatives of hydroxyphenyl-urethane, have been prepared and proposed as remedies by E. Merck.

Urea and dulcine are the only remaining members of the group which require further consideration.

Urea. Carbamide.

$$CH_4ON_2 ; \textit{i.e.,}$$

$$C=O \begin{cases} NH_2 \\ NH_2 \end{cases}$$
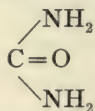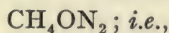
Urea exists in the urine of mammals, and in blood, milk, and other animal fluids.   In the circulating fluids and tissues, urea exists in the merest traces, there is normally no threshold value for urea in the kidney; the blood is practically freed of urea by passing through the kidney.   The urea is formed largely in the liver, not at all in the kidney apparently.   It is also eliminated to some extent in the stools and perspiration, and with free natural or forced sweating relatively large amounts of urea may be thus removed.   It was first obtained synthetically by Wöhler in 1828, being the first of the natural organic bodies prepared by a synthetic process.

Urea may be prepared by a variety of methods, of which the following are the most important and interesting:

1. Fresh urine is concentrated at 100° to one-tenth of its volume, and the insoluble deposit of phosphates and urates separated by filtration.   The filtrate is mixed with an equal volume of a hot concentrated solution of oxalic acid, and the whole vigorously agitated and allowed to cool.   A copious, fawn-coloured precipitate of oxalate of urea is obtained, which is separated by a filter, slightly washed with cold water and pressed.   The product is dissolved in boiling water, and powdered chalk added till the liquid becomes neutral and effervescence ceases.   The liquid is filtered from the calcium oxalate, warmed with animal charcoal, filtered, and concentrated by evaporation, avoiding actual boiling.   The urea which deposits on cooling is purified by recrystallisation.

2. Liebig and Wöhler's classical method of preparing urea affords an interesting example of rearrangement of the atoms in the molecule. Both ammonium cyanate and urea have an elementary composition corresponding to the empirical formula: CH_4ON_2.   On evaporating an aqueous solution of ammonium cyanate at the temperature of boiling water, the salt suffers molecular change into urea, according to the

equation: $CN.O(NH_4) = CO(NH_2)_2$. The conversion is never quite complete.

In carrying out Liebig's reaction in practice, it is not necessary to operate on pure ammonium cyanate. Potassium cyanate in strong aqueous solution is treated with an equal weight of ammonium sulphate, and the whole evaporated to dryness on the water-bath. The product is boiled with strong alcohol, which dissolves the urea, leaving a residue of potassium and ammonium sulphates. On concentrating and cooling the alcoholic solution, crystals of urea are deposited. Instead of employing potassium cyanate previously prepared, it may be extemporised by heating a mixture of 28 parts of dehydrated potassium ferrocyanide and 14 parts of manganese dioxide in an iron vessel till it becomes sticky. The product is extracted with cold water, evaporated to dryness with 20.5 parts of ammonium sulphate, and the residue extracted with alcohol as before.

J. Williams (*Jour. Chem. Soc.*, **21**, 63) proposed the use of lead cyanate in place of the potassium salt.[1] It is digested with water and an equivalent quantity of ammonium sulphate at a gentle heat, the liquid filtered from the insoluble lead sulphate, and the filtrate evaporated to the crystallising point.

Fig 13.—Urea: *a*, quadrilateral prisms; *b*, indefinite crystals, as deposited from alcoholic solutions.

3. Urea has been obtained by passing a current of air mixed with ammonia and benzene vapour over heated platinum wire (*Jour. Chem. Soc.*, **39**, 471), and by passing ammonia and carbon dioxide through a red-hot tube.

Urea forms transparent, colourless, four-sided, somewhat hygroscopic anhydrous prisms (Fig. 13). It is odourless and possesses a cooling saline taste, like that of nitre. When heated to 132° urea melts, and at 150 to 160° decomposes with evolution of ammonia and

[1] The lead cyanate is prepared by fusing the best commercial cyanide of potassium at a very low red heat in a shallow iron vessel, and gradually adding red lead, in small quantities at a time, with constant strring, so as to avoid much rise of temperature. The product is poured out, finely powdered, exhausted with successive portions of cold water, the solution filtered, and barium nitrate added. The liquid is filtered from the precipitate of barium carbonate, and treated with lead nitrate. The precipitated lead cyanate is washed thoroughly and dried at a gentle heat.

formation of biuret, $C_2H_5O_2N_3$,[1] which on further heating splits into ammonia and ammonium cyanate, leaving a residue containing melanuric acid, $C_3N_3(OH)_2NH_2$, and cyanuric acid, $C_3H_3O_3N_3$, which bears a much stronger heat without change.[2] In a vacuum, urea distils unchanged at 135°.

Urea is soluble in an equal weight of cold water, and in a much less quantity at 100°. It is also readily soluble in alcohol, and dissolves in amyl alcohol, but it is nearly insoluble in ether, and quite so in chloroform and volatile oils.

At the ordinary temperature, an aqueous solution of pure urea has practically no tendency to change, but on boiling, a certain reversion to ammonium cyanate takes place. The transformation ceases in about an hour, when the decomposition is between 4 and 5% (Walker and Hambly, *J. Chem. Soc.*, **67**, 749). When heated with water under pressure, urea undergoes hydrolysis, with formation of ammonium carbonate: $CH_4ON_2 + 2H_2O = (NH_4)_2CO_3$.

Many bacteria ferment urea, with the production of ammonium

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[1] **Biuret**, $NH:(CO.NH_2)_2$, is formed when urea is heated to 150–160°, until the fused substance becomes pasty and ceases to evolve ammonia. On treating the product with hot water, cyanuric acid remains undissolved, and biuret crystallises out on concentrating and cooling the filtrate. It may be purified by re-solution in hot water and precipitation with dilute ammonia.

Biuret crystallises from water in long, white, acicular crystals, containing 1 H_2O or from alcohol in anhydrous laminæ. It is sparingly soluble (1:65) in cold water, but very readily (45:100) in boiling water, and is easily soluble in alcohol. Biuret is dissolved unchanged by cold concentrated sulphuric acid.

Biuret in aqueous solution is not precipitated by tannin, nor by solutions of lead or silver. Its most characteristic reaction is the production of a red or violet solution (the tint varying with the relative proportions of biuret and the reagent employed), on adding sodium hydroxide and a few drops of a solution of cupric sulphate or Fehling's solution. This test, often referred to as "the biuret reaction," affords a valuable means of detecting urea (compare page 293).

Biuret is a weak base, forming salts readily decomposed by water. The cyanurate, $B,C_3H_3O_3N_3$, is deposited in needles during the preparation of biuret. It differs from urea cyanurate, for which it has been mistaken, by giving $3NH_3$ instead of $2NH_3$ when boiled with baryta-water, and in evolving 14.8% of nitrogen instead of 11.5% when treated with alkaline hypobromite.
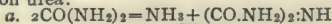
When heated to a temperature above 170°, biuret is decomposed into ammonia and cyanuric acid. Heated in a current of hydrochloric acid gas, it yields cyanuric acid, urea, and guanidine, together with ammonia and carbon dioxide:
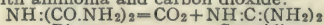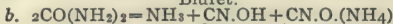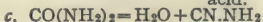
$$NH:(CO.NH_2)_2 = CO_2 + NH:C:(NH_2)_2$$
$$\text{Biuret.} \qquad\qquad \text{Guanidine.}$$

[2] Drechsel (*Jour. pr. Chem.* [ii], **9**, 284) gives the following formulæ in illustration of the action of heat upon urea:
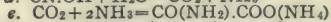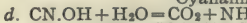
$$a. \quad 2CO(NH_2)_2 = NH_3 + (CO.NH_2)_2:NH$$
$$\text{Biuret.}$$

$$b. \quad 2CO(NH_2)_2 = NH_3 + CN.OH + CN.O.(NH_4)$$
$$\text{Cyanic} \quad \text{Ammonium}$$
$$\text{acid.} \quad\;\; \text{cyanate.}$$

$$c. \quad CO(NH_2)_2 = H_2O + CN.NH_2$$
$$\text{Cyanamide.}$$

$$d. \quad CN.OH + H_2O = CO_2 + NH_3$$

$$e. \quad CO_2 + 2NH_3 = CO(NH_2).COO(NH_4)$$
$$\text{Ammonium carbamate.}$$

$$f. \quad 2CN.OH. + CN.NH_2 = C_3N_3(OH)_2NH_2$$
$$\text{Melanuric acid.}$$

$$g. \quad 3CN.OH = C_3N_3(OH)_3$$
$$\text{Cyanuric acid.}$$
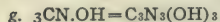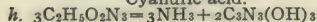
$$h. \quad 3C_2H_5O_2N_3 = 3NH_3 + 2C_3N_3(OH)_3$$
$$\text{Biuret.} \qquad\quad \text{Cyanuric acid.}$$

carbonate. This occurs in pure solutions of urea, as well as in mixtures containing organic matter. This decomposition occurs often in the bladder, as the result of septic infection, ammoniacal reaction and precipitation of the calcium phosphates and possibly urates occurring in consequence. This bacterial decomposition occurs very easily in urine, since all urine is infected on being voided, though sterile in the normal bladder. For the preservation of urine, therefore, it is necessary to add an antiseptic, as thymol, chloroform or toluene, or to refrigerate.

Urea also yields ammonia when fused with alkali hydroxide or ignited with soda-lime, a carbonate being formed at the same time. When heated with a strong mineral acid, urea similarly forms an ammoniacal salt, carbon dioxide being evolved.

When sterile, urea remains unchanged in slightly acid solution; but if the solution contain a fixed alkali, the urea will be slowly converted into ammonium carbonate.

Pure concentrated nitric acid combines with urea without decomposing it, but if the acid contain nitrous acid the urea is resolved into water, nitrogen, and carbon dioxide, according to the following equation: $CH_4ON_2 + N_2O_3 = 2H_2O + 2N_2 + CO_2$. With Millon's reagent the reaction occurs promptly and completely, and may be employed for the determination of urea. Chlorine, bromine, hypochlorites, and hypobromites decompose solutions of urea with evolution of nitrogen.

A compound of urea with sodium chloride, of the formula CH_4ON_2, $NaCl,H_2O$, separates in brilliant rhombic crystals when mixed solutions of urea and common salt are evaporated. This compound sometimes crystallises from concentrated human urine.

Salts of Urea.

Urea is a somewhat feeble base. It forms a well-defined series of salts, all of which are more or less soluble. Many of them are decomposed by excess of water, and the aqueous solutions are in all cases acid to litmus as the result of this hydrolytic dissociation. The *nitrate* and *oxalate* of urea crystallise well, and are employed for the isolation and detection of urea. Urea also combines with metallic salts, the compounds being mostly soluble, with the exception of those with mercuric nitrate.

Urea Nitrate, CH_4ON_2,HNO_3, separates in crystals when mod-

erately strong nitric acid is added to a concentrated aqueous solution
of urea, and the liquid cooled. The compound forms brilliant white
scales or plates, or, if the deposition is slow, prismatic crystals. When
nitric acid and urea are brought together on a microscope-slide, and
the reaction observed under a low power, the formation of obtuse
rhombic octahedra is first noticed, the angles being *constantly* 82°.
These octahedra change to rhombic and hexagonal tables, either sepa-
rate or superposed (see Fig. 14, *a*), but also having angles of 82°. For
the formation of nitrate of urea from normal urine, it is sufficient to
concentrate the liquid to about one-fourth of its volume, filter after
cooling from the precipitated urates, etc., and add nitric acid to the
cold filtrate. Nitrate of urea is not changed in the air. It is readily
soluble in water, forming a solution of acid reaction and taste. It is
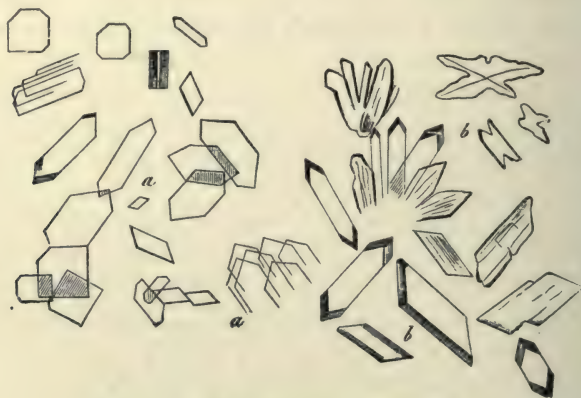
Fig. 14.—*a*, Urea Nitrate; *b*, Urea Oxalate.

also soluble in alcohol, but very slightly soluble in presence of nitric
acid. Oxalic acid precipitates urea oxalate from concentrated solu-
tions of the nitrate.

Urea Oxalate, $(CH_4ON_2)_2,C_2H_2O_4$, is readily formed on mixing
concentrated solutions of urea and oxalic acid. From urine it may
be prepared by adding oxalic acid to the concentrated and filtered
liquid. Urea oxalate forms thin crystalline plates (see Fig. 14, *b*),
usually grouped together, but sometimes in well-formed separate crys-
tals. Its microscopic appearance is not unlike that of the nitrate of
urea, but the forms are less characteristic, and the angles are different.
Oxalate of urea is soluble with difficulty in cold water, but dissolves

readily at a boiling heat. It is less soluble in a solution of oxalic acid than in pure water. The salt dissolves in 62 parts of alcohol, but is quite insoluble in amyl alcohol. Hence, if a solution of urea in amyl alcohol (such as will result from evaporating urine to dryness, heating the residue with amyl alcohol, and filtering) be treated with a cold saturated solution of oxalic acid in amyl alcohol, urea oxalate will be precipitated in small crystals (see further, page 295).

The *hydrochloride* B,HCl is a very deliquescent crystalline mass, formed by the action of hydrogen chloride on urea. It is decomposed by water into its constituents. $B,HAuCl_4, + 1\ H_2O$ forms orange-red prisms or needles, very soluble in water, alcohol, and ether. $B_2,H_2PtCl_6,2H_2O$ forms yellow needles, very soluble in hot water. B,H_3PO_4 is obtained in large, very soluble, rhombic crystals on evaporating pig's urine or mixed solutions of urea and phosphoric acid.

Detection of Urea.

Urea produces no precipitate with tannin or other general reagents for the alkaloids. It gives no reaction with either neutral or basic lead acetate, and does not reduce Fehling's solution even on boiling. It gives no colour-reactions with oxidising agents.

If a fragment of solid urea be moistened with a concentrated solution of furfural, and a drop of strong hydrochloric acid (sp. gr. 1.10) be then added, a fine violet colouration is produced (Schiff, *Ber.*, **10,** 774).

If a residue containing urea be heated for some time to a temperature not exceeding 160°, the product will contain biuret. On dissolving it in water, adding sodium hydroxide, and then dropping in a dilute solution of cupric sulphate, a violet or red colouration will be produced if urea were originally present.

If an aqueous solution of urea be heated with silver nitrate, a white precipitate of silver cyanate is formed, soluble in boiling water, while the filtered liquid is found to contain ammonium nitrate: $CO:N_2H_4 + AgNO_3 = CN.AgO + NH_4NO_3$.

For the recognition of urea in dilute aqueous solution Bloxam has suggested the following method: If a nitrate be present, add a few drops of ammonium chloride solution, but if absent, acidify the liquid with hydrochloric acid. Evaporate the solution to dryness in a watch-glass, and heat the residue cautiously as long as thick white

fumes are evolved. Dissolve the cooled residue in a drop or two of ammonia, add a drop of barium chloride, and stir. If urea were present, crystalline streaks of barium cyanurate will be formed in the track of the glass rod.

When sulphuric acid is added to a solution of sodium nitrite containing urea, colourless nitrogen and carbon dioxide are evolved; in the absence of urea, the fumes are yellow-brown.

The test of Lüdy may sometimes be usefully employed. The material to be tested for urea is concentrated at faintly acid reaction, and the residue extracted with alcohol. This extract is mixed with an excess of a solution of o-nitro-benzaldehyde in alcohol, and the mixture evaporated to dryness on the water-bath. The residue is repeatedly extracted with alcohol, until the extract gives no reaction with phenyl-hydrazine. The final residue contains a condensation product, nitro-benzilydenediureide, that clings tenaciously to the sides of the beaker. This residue is covered with a solution of phenyl-hydrazine hydrochloride, a few drops of dilute sulphuric acid added, and the mixture boiled. The presence of urea is indicated by the development of a red colour, due to the phenyl-hydrazone of o-nitro-benzaldehyde.

On mixing a solution of urea with one of neutral mercuric nitrate, a white flocculent precipitate is obtained. This has a composition dependent on the concentration of the liquid, containing, according to the conditions of its formation, 1, 1.5, or 2 molecules of mercuric oxide to 1 of urea. If, however, the addition of the mercuric nitrate be continued as long as precipitation occurs, and sodium hydrogen carbonate be added in quantity sufficient to neutralise the nitric acid set free, the precipitate has the composition $CH_4ON_2,2HgO$. The end of the action is indicated by the yellow colour developed from the formation of basic nitrate of mercury. The mercuric oxide compounds of urea are decomposed by hydrogen sulphide with precipitation of mercuric sulphide and liberation of urea, and the procedure may be utilised for the isolation of the base from urine.

Urea is not precipitated by a solution of mercuric chloride. The addition of mercuric nitrate to a soluble chloride results potentially in the formation of mercuric chloride. As sodium chloride is present in urine, mercuric nitrate produces no precipitate of Liebig's compound in that liquid until sufficient has been added to react fully with the chloride present.

Mercuric acetate gives no precipitate with urea in the cold, and the separation is very incomplete on boiling.

The recognition of urea in animal fluids is usually based on the preparation of the nitrate or oxalate. If the quantity of urea present is sufficient for the preparation of these salts in such amount as to allow a study of their properties, the determination of urea can be effected. On the other hand, if the quantity of urea present be very minute, as in the case of blood and of all secretions and excretions other than urine, it is not always easy to avoid error.

For the detection of urea in blood-serum or other serous fluids, the liquid should be mixed with three or four volumes of alcohol, which precipitates the albuminous matters.[1] The filtered liquid is evaporated on the water-bath, and the residue exhausted with absolute alcohol. The alcoholic solution is evaporated on a watch-glass, and if foreign matters show themselves, the treatment with absolute alcohol is repeated. The extract is evaporated nearly to dryness on a watch-glass, the residue taken up with water, and any phosphates precipitated by addition of barium hydroxide solution. Carbon dioxide is passed through the filtered liquid, which it then boiled, again filtered, and evaporated on the water-bath to a syrup. The residue is divided into 2 or 3 portions, which are treated respectively with nitric acid and with oxalic acid, and the products examined under the microscope for the recognition of the characteristic crystalline forms of urea nitrate and oxalate, as shown in Figs. 14 a and 14 b (page 292).

In carrying out the foregoing process, it is very important to study carefully the crystals supposed to be urea nitrate, and, whenever · possible, to dissolve and test them with mercuric nitrate. Under certain conditions, and especially in presence of extractive matters, one may meet with nitrates of alkali-metals which resemble in their microscopic appearance the crystals of urea nitrate. The inorganic salts are distinguished from the latter by their behaviour on ignition, and by the presence of a notable quantity of potassium or sodium in the ash, which will have an alkaline reaction. On the other hand, if a crystal of true urea nitrate be dissolved in water and treated with a concentrated solution of oxalic acid (which may be effected under the microscope), crystals of urea oxalate will be gradually formed.

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[1] In some cases it is desirable to effect a preliminary separation of the bulk of the proteins by acidifying the liquid with acetic acid and boiling.

The value of oxalic acid as a reagent for the isolation and recognition of urea is considerably enhanced if advantage be taken of the sparing solubility of urea oxalate in a mixture of alcohol and ether. A still better method is to heat the alcoholic extract to be tested with a small quantity of amyl alcohol, and then treat the solution, decanted or filtered if necessary, with a cold saturated solution of oxalic acid in amyl alcohol. The urea oxalate is precipitated in small crystals, which redissolve on warming the liquid, and on cooling separate out in a condition suitable for microscopic examination. The process may be modified by treating the solution of urea in amyl alcohol with one of oxalic acid in anhydrous ether. Precipitation takes place abundantly and quickly, but the crystals are usually small and imperfect. The oxalic acid may be added in powder, the liquid heated and thoroughly cooled, and the excess of oxalic acid removed from the precipitate by treatment with anhydrous ether. The method is capable of being employed quantitatively. The amyl alcohol used in the process must not develop a red or brown colour with oxalic acid, and should be free from water and ethyl alcohol.

Estimation of Urea.

The estimation of the urea contained in urine is often of great physiological and pathological interest.

From 85 to 90% of the total nitrogen contained in normal human urine exists in the form of urea, the remainder being divided between ammonia, amino-acids, uric acid, hippuric acid, purine bases, creatinine, etc. In the urine of herbivorous mammals the uric acid is replaced by hippuric acid, while the nitrogen of birds and reptiles is eliminated chiefly in the form of uric acid instead of as urea.

As urea is the predominant nitrogenous constituent of normal human urine, it is evident that for many purposes its estimation will afford sufficient information as to the amount of nitrogen passing away in the urine. There has been in the past much misconception as to the relations of urea to the other urinary nitrogenous compounds, and the older figures are relatively worthless. Urea represents the end-product (with ammonia) of the common metabolism of protein, both endogenous and exogenous; the eliminations of purines and of creatinine are independent, except that probably a small fraction of each is oxidised to urea. The relation of ammonia to urea in the urine is the expression of acidosis, i.e., when acids are eliminated they withdraw

ammonia from the conversion to urea. Since the urea represents the end product of the metabolism of common protein, the urea output will vary with the protein of the diet. With a low protein diet, the urea output may fall to as low as 10 grm. per day, 60 % of the nitrogen only being in the state of urea. With high input of protein in the diet, the urea output may be as high as 75 or more grm. per day, or over 95 % of the total nitrogen. Obviously therefore the relation of urea-N to total-N in the urine is of no value unless the protein input is known, and the intestinal N-output as well. There is no purpose in making a urea estimation unless the nitrogen input of the subject is accurately known and controlled, and the fœcal nitrogen as well.

The *amount* of urea excreted in the urine varies considerably with the diet, being increased by nitrogenous foods. The weight of urea excreted per diem by an adult man on mixed diet ranges from 15 to 40 grm., the average being about 33 grm. (500 grains). On a diet poor in protein the excretion of urea may fall to 15 to 20 grm., while on a flesh diet the daily output may rise to 100 grm. The proportion of urea in human urine averages about 2 %, but dog's urine is stated to contain 10 %. This varies with the water output.

A large excretion of urea, if long continued, points to *increased tissue-metabolism* or to surplus nitrogenous ingesta, but a temporary increase may be simply due to increased urination. Similarly, diminished excretion of urea may be due to diminished metabolism or to retention of urea in the system.

A great number of methods have been devised for the estimation of urea, and of these only the following deserve notice:

1. The precipitation of urea in the form of oxalate is a convenient way of isolating the base from complex mixtures, and under certain conditions gives very fair results. The best method of applying it is described by Gottleib (*Arch. f. Exper. Path.*, 1908, **42**, 328).

The blood or pressed extracts of tissues, which must be fresh and display no alkaline reaction, are mixed with 4 volumes of alcohol and filtered after 24 hours, the precipitate well washed with 65 % alcohol and the collected filtrates concentrated, after acidification with acetic acid, under diminished pressure at 50°. The residual fluid is then extracted with chloroform, to remove all lipoids, the chloroform washed with water and the water added to the solution. The concentration under diminished pressure at 40° is then continued until the alcohol is largely removed. Then the residue is acidified with sulphuric acid

to 5%, and a solution of 10% phosphotungstic acid in 5% sulphuric acid added so long as a precipitate forms. The filtrate and wash water are freed of sulphuric acid and phosphotungstic acid by the careful addition of barium hydroxide solution, the excess of barium removed with carbon dioxide. The urea is then precipitated from the faintly acidified solution by the addition of mercuric nitrate. The precipitate is decomposed with hydrogen sulphide, the insoluble sulphide removed by filtration, the excess of the hydrogen sulphide removed with a stream of air, the solution neutralised by the careful addition of barium hydroxide solution, and any excess of the latter removed with carbon dioxide, and the final filtrate concentrated under diminished pressure at 40°. The residue is twice taken up in absolute alcohol and concentrated as before. The final residue is taken up in absolute alcohol, and a concentrated ethereal solution of oxalic acid added in slight excess. The precipitated urea oxalate is collected on a filter paper, washed with ether until free from oxalic acid. There is a solubility of about 1/10 mm. to the 10 c.c. of washing ether. The dried urea oxalate may be weighed; the oxalic acid may be titrated with $N/10$ barium hydroxide solution; or an estimation of nitrogen may be made. The results will usually fall too low, but the method is the best at our command. According to a recent suggestion of Folin, the urea may be converted into ammonia, and the latter estimated by the use of Nessler's reagent, as in the estimation of ammonia in water, with a high degree of accuracy when dealing with amounts of urea so small as to make the usual methods of estimation fallacious.

2. Urea is converted into ammonia when heated with strong sulphuric acid. The reaction having been effected, the ammonia may be estimated by any known method. Thus, it may be distilled off after addition of sodium hydroxide or lime, and the distillate titrated with standard acid; this is the Kjeldahl method for the estimation of urea. The hydrolysis of urea to ammonia occurs, however, on heating in water alone, as discovered by Bunsen. Upon the direct hydrolysis of urea by heat (the reverse of the reaction whereby in the body urea is formed from ammonium carbonate) are based the modern methods for the estimation of urea that have supplanted all the earlier methods. The problem has been to accomplish the complete conversion of the urea to ammonia, without converting any other of the nitrogenous components of urine into ammonia, which would exaggerate the results. This may be accomplished in one of two ways:

either by a preliminary separation of the urea; or much better, by so arranging the conditions of the estimation as to exclude or reduce to a negligible minimum the hydrolysis of other substances. The estimation of urea according to the last method is accurately and conveniently accomplished by the methods of Folin and of Benedikt, and these alone will be described.

The method of Folin is based upon the fact that crystalline magnesium chloride ($+6$ mol. H_2O) melts at $115°$, and boils at about $155°$. If urea is contained in this melted salt, it is split into ammonia and carbon dioxide. Acid must be provided to combine with the ammonia. To carry out the test, 5 c.c. urine are placed in a 200 c.c. Erlenmeyer flask, 20 grm. of purest magnesium chloride added, a small piece of paraffin, a drop of alizarin red solution and 2 c.c. of hydrochloric acid. Into the neck of the flask is inserted a stopper bearing a special form of safety reflux condenser, with three bulbs. The mixture is then heated over the free flame until the excess of water is distilled off, and the drops of distillate from the condenser produce a sputtering. Then the temperature is cut down by reduction of the flame and the heating continued for one hour. There are two precautions to be taken. The flask must not bump; this is to be prevented by regulation of the heat. The contents must not become alkaline; this is prevented by carefully shaking back a few drops of the acid distillate, as needed, that is collected in the balls of the safety condenser. The hot solution is then transferred to a half-litre flask, care being taken to save all the fluid in the condenser. It is well to avoid an excess of water in the transfer. The solution is then made alkaline (5 c.c. concentrated potassium hydroxide) and distilled into a known volume of $N/10$ acid. The distillation must be prolonged until the flask is almost dry, otherwise there will be a deficit. From the ammonia bound by the $N/10$ acid, the amount in the 5 c.c. of urine is calculated, the preformed ammonia, determined in direct estimation, being, of course, subtracted.

This method gives very good results. There are, however, certain defects. The prolonged distillation, necessary to drive over all the ammonia, breaks up small amounts of creatinine and uric acid, possibly of hippuric acid. The necessity of keeping the contents of the digestion flask acid makes constant supervision necessary. The best magnesium chloride contains ammonia, and a blank must be made and a subtraction effected. These defects can be largely avoided as follows. Lithium chloride, which can be obtained free from

ammonia, should be employed instead of magnesium chloride.   This
has the further advantage that it does not hold the ammonia back
in the distillation as does magnesium chloride, so that one does not
need to distil so long.   Supervision of the reaction remains however
necessary.   With the use of the lithium chloride it becomes possible to
secure the ammonia without any heating, if the urine contain other
substances unstable at alkaline reaction and especial accuracy is
desired.   This is accomplished, as suggested by Gill, Allison and
Grindly, by rendering the solution alkaline with hydroxide or car-
bonate, and removing the ammonia by a stream of air, as in the Folin
method for the estimation of ammonia.

Under certain circumstances, when the urine contains sugar or is a
pathological urine that cannot be trusted not to react with the forma-
tion of ammonia, a purification of the urine becomes necessary.   In
the estimation of urea in materials other than urine, such a purification
will usually be necessary.   There are two methods of purification: by
barium hydroxide and by phosphotungstic acid.

When the urine contains sugar or other carbohydrates, it should be
purified by the addition to 10 c.c. urine of 3 grm. of powdered
barium hydroxide.   This is well shaken, then 200 c.c. of alcohol-ether
(2:1) added, the mixture well shaken and set aside until the following
day.   Filtration is then made, and 100 c.c. of the filtrate taken
(corresponding to 5 c.c. urine) and evaporated under diminished
pressure at not over 55° to about 25 c.c.; an equal amount of water
is added and a little magnesium oxide and the concentration con-
tinued until the volume is reduced to about 5 c.c.   This operation,
which drives off the preformed ammonia and renders a correction for
ammonia unnecessary, is best carried out in the 200 c.c. flask in
which the estimation is to be made according to the Folin method.
When the concentration is completed, the estimation is finished.

A better method for purification, in the absence of sugar, is by
precipitation with phosphotungstic acid.   The reagent is a 10%
solution in 5% sulphuric acid; it must be ascertained that the
phosphotungstic acid used does not precipitate urea.   For orien-
tation, it is necessary to find out for every particular urine, how much
of this solution is necessary for complete precipitation, as excess is to
be avoided.   This having been determined, 50 c.c. of urine are
precipitated with the necessary amount of the reagent, the flask filled to
the mark with $2N$ H_2SO_4 and after a short time filtered, each 10 c.c.

of the filtrate being equal to 5 c.c. urine. This solution can be used directly in the autoclave method later to be described. For use with the Folin method, 10 c.c. are rubbed up in a dish with calcium hydroxide to precipitate the sulphuric acid and the phosphotungstic acid. When the mixture is alkaline, it is filtered, washed, and the filtrate used according to the method of Folin. Sometimes in the process of neutralisation with the lime, a blue colour develops; filtration cannot be performed until this has faded.

More convenient than the method of Folin is that of Benedikt, and it is possibly more accurate, at least in that the duplicates agree more closely, and the method requires no supervision during the period of heating. 5 c.c. of urine (if necessary, purified according to the stated methods) are placed in a long and rather wide heavy testtube, and 3 grm. of potassium hydrogen sulphate and 2 grm. of zinc sulphate added, with a small piece of paraffin. This tube is then immersed in a bath of sulphuric acid or paraffin at 160° for an hour. The mixture foams at first, but if the tube be long, there is no danger of frothing. When the heating is completed, the contents are allowed to cool down to below the b. p., then diluted with water, and distilled over into $N/10$ acid solution as before. The distillation does not need to be continued so long as in the case of the original Folin method. The ammonia may also be driven over in the cold by the use of a stream of air, as described. In the experience of the writer, this method has proved very satisfactory. A correction is required of course for the preformed ammonia, but none for the reagents.

In laboratories where an autoclave is available, urea may be estimated by direct heat under pressure, and this is a valuable method when many estimations are to be made. The urine is first precipitated with phosphotungstic acid as previously described, 10 c.c. of the filtrate placed in a test-tube, covered with tin foil and heated in the autoclave at 150° for an hour and a half. The ammonia is then either distilled into $N/10$ acid or transferred with a current of air, and the result corrected for preformed ammonia. With one or other of these methods, in one or other of the stated modifications, the estimation of urea can under all circumstances be accurately accomplished.[1]

[1] A method of estimating urea based on the use of *urease* may here be suggested. Takeuchi (*J. Coll. Agric. Univ. Tokyo*, 1909, 1, 1-14), has shown that this enzyme which hydrolyses urea to carbon dioxide and ammonia, is present in the Soja bean in considerable quantity.

Para-phenetole-carbamide.　Dulcine.　Sucrol.

$$\begin{array}{l} \diagup NH.C_6H_5.+OEt \\ C=O \\ \diagdown NH_2 \end{array}$$

This substance has a pure sweet taste, about 200 times as intense as that of cane-sugar, and is now employed as a substitute for sugar in cases where the use of sugar is physiologically objectionable.

Dulcine was first prepared by the reaction of paraphenetidine hydrochloride (Vol. VI, p. 98) with potassium cyanate, but has been subsequently obtained by the reaction of p-phenetidine with carbonyl chloride, and treatment of the resultant chloro-compound with ammonia:[1]

Dulcine has also been prepared by the reaction of p-phenetidine with aniline, when it is produced together with diparaphenetole carbamide. The taste of the latter compound is not sweet, but by heating for several hours to 160° with an equivalent quantity of urea it is converted into the monophenetole derivative (dulcine), according to the equation:

$$CO:(NH.C_6H_4.OC_2H_5)_2 + CO(NH_2)_2 = 2(NH_2.CO.NH.C_6H_4.OC_2H_5).$$
Diparaphenetol-carbamide.　Urea.　　　　　Dulcine.

Dulcine is also produced by heating the diphenetole-urea with ammonium carbamate or commercial carbonate of ammonium.

Dulcine forms colourless or yellowish shining needles, which melt at 173 to 174°. It is soluble in about 800 parts of cold or in 50 of boiling water, and dissolves in 25 parts of rectified spirit. It is also

Armstrong and Horton (*Proc. Roy. Soc.*, 1912, **85** B, 109) have studied its quantitative action on urea and substituted ureas by digesting the solution of the urea with the enzyme, subsequently neutralising the ammonia formed by an excess of $N/10$ acid, boiling, to expel carbon dioxide and finally titrating back with barium hydroxide. In view of the fact that the action of urease is perfectly selective, other substances such as alanine, allantoin, arginine, benzamide, glycocoll, guanine, hippuric acid, histidine, creatinine, leucine, tyrosine and uric acid being unaffected, a method such as described above, would probably prove valuable for estimating urea in presence of the above or similar substances. The carbon dioxide formed by the urease could also be measured or weighed and serve as a means of estimating urea.—*W. A. D.*

[1] A solution of p-phenetidine in benzene is gradually added to a 20 % solution of carbon oxychloride in the same solvent. After standing for an hour or so, the liquid is filtered, and the filtrate treated with ammonia gas or shaken with a strong solution of ammonia. The ammonium chloride is filtered off and the filtrate evaporated, the residue washed with cold water, and the dulcine recrystallised from boiling water.

If concentrated solutions are employed in the foregoing process diparaphenetole-carbamide is also formed. According to F. v. Heyden, in operating on a large scale, the carbonyl chloride compound is not formed, or only in small amount, the reaction taking place with formation of paraethoxyphenyl isocyanate, $CO:N.C_6H_4.OEt$, which body yields dulcine on treatment with ammonia.

　a. $2NH_2.C_6H_4.OC_2H_5 + COCl_2 = NH_2.C_6H_4.OC_2H_5,HCl + COCl.NH.C_6H_4.OC_2H_5$
　　p-phenetidine.
　b. $COCl.NH.C_6H_4.OC_2H_5 + 2NH_3 = NH_4Cl + NH_2.CO.NH.C_6H_4.OC_2H_5$
　　　　　　　　　　　　　　　　　　　Dulcine.

Dulcine is also obtained by heating p-phenetidine with urethane or with acetyl-urea.

soluble in ether and in benzene. When pure it dissolves in concentrated sulphuric acid without colouration. When boiled with water, dulcine gradually decomposes into ammonium carbonate and diparaphenetole-carbamide.

On adding fuming nitric acid to a fragment of solid dulcine a violent reaction occurs, and the substance dissolves with orange-red colouration. On evaporating the liquid to dryness at 100°, an orange-yellow resinous substance is obtained, which is soluble in ether, chloroform, or alcohol. If this resinous product be triturated with a mixture of equal parts of phenol and strong sulphuric acid the colour changes to a blood-red, which is permanent for a considerable time.

For the detection of dulcine in wine, beer, etc., G. Morpurgo treats the suspected liquid with 5 % of lead carbonate, evaporates on a water-bath to a thick paste, and treats the residue several times with strong alcohol. The alcoholic solution is evaporated to dryness, and the residue extracted with ether. On evaporating the ether, a residue is obtained of nearly pure dulcine, which may be recognised by its sweet taste and by the following reaction. The residue is warmed with 2 drops of phenol and 2 drops of concentrated sulphuric acid, the resultant reddish-brown syrup rinsed into a test-tube with a few c.c. of water, and the liquid cooled. Ammonia or sodium hydroxide is then poured cautiously on to the surface, when the production of a blue or violet-blue zone at the junction of the two layers will indicate the presence of dulcine.

IMINO BASES.

The imino bases are distinguished by containing the group—NH, otherwise than as a link in a closed chain, as it exists in xanthine, uric acid, etc. In some cases, as in that of guanidine, the imino bases also contain one or more amino groups. The members of the group have in some cases considerable interest, and certain of them are important constituents of meat-juice and other animal products; but none of them have hitherto received any practical application in an isolated state. The chief members of the class may be thus formulated:

Guanidine.

$$NH{:}C\left\{\begin{array}{l}NH_2\\NH_2\end{array}\right.$$

Methyl-Guanidine.

$$NH{:}C\left\{\begin{array}{l}NH_2\\NH.CH_3\end{array}\right.$$
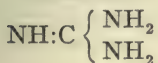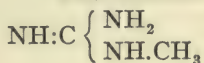
Glycocyamine.[1]
(Guanido-acetic acid.)
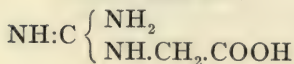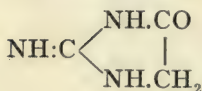
$$NH:C \begin{cases} NH_2 \\ NH.CH_2.COOH \end{cases}$$

Creatine.
(Methyl-Glycocyamine.)

$$NH:C \begin{cases} NH_2 \\ NCH_3.CH_2.COOH \end{cases}$$

Glycocyamidine.[2]

$$NH:C \begin{cases} NH.CO \\ | \\ NH.CH_2 \end{cases}$$

Creatinine.
(Methyl-Glycocyamidine.)

$$NH:C \begin{cases} NH.\ CO \\ | \\ NMe.CH_2 \end{cases}$$

Guanidine.   Iminourea.

$$CH_5N_3; \text{ } i.\text{ } e.,$$

$$C \begin{cases} NH_2 \\ =NH \\ NH_2 \end{cases}$$
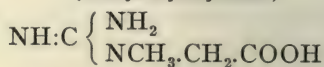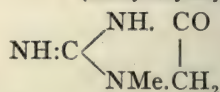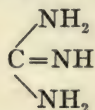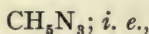
Guanidine has not been detected in any animal tissue or fluid, but has been isolated from vetch-seedlings. It has been obtained by the direct oxidation of proteins, and yields urea by boiling with barium hydroxide solution or dilute acids. Guanidine is the chief product of the action of oxidising agents on guanine, and may be regarded as a connecting link between creatine and the purine bases. While guanidine itself does not occur in the animal body, very important guanidine derivatives occur in the tissues and secretions.

Guanidine has been obtained synthetically by several methods, among which may be mentioned the reaction of cyanamide with ammonia: $CN.NH_2 + NH_3 = NH:C(NH_2)_2$. In practice, ammonium chloride is heated with an alcoholic solution of cyanamide.

Guanidine may be conveniently prepared by heating dry ammonium thiocyanate to 180–190° for 20 hours. A portion of the thiocyanate is isomerised into thiourea, which reacts with the undecomposed ammonium thiocyanate to yield a product consisting mainly of guanidine thiocyanate. This is purified by crystallisation from water or alcohol, and the solution of 100 parts mixed with the solution of 58 parts of potassium carbonate, and evaporated to dryness. From the residue, the potas-

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[1] **Glycocyamine** is obtained when glycocoll is boiled with guanidine carbonate in aqueous solution, ammonium carbonate being simultaneously formed. It forms transparent needles, sparingly soluble in cold water, but readily on boiling, and insoluble in alcohol. Boiled with cupric acetate it gives microscopic crystals containing $Cu(C_3H_6O_2N_3)_2$. According to A. B. Griffiths, glycocyamine occurs in putrid flesh.

[2] **Glycocyamidine** hydrochloride is obtained when glycocyamine hydrochloride is heated to 160°. On boiling the product with water and lead hydroxide the free base is obtained in laminæ of alkaline reaction, which have a bitter taste, are poisonous, and very soluble in water. Glycocyamidine forms a compound with zinc chloride which crystallises in needles closely resembling the corresponding compound of creatinine.

sium thiocyanate is removed by treatment with alcohol, and the guanidine carbonate recrystallised from water. From this salt free guanidine is obtained by dissolving it in the calculated quantity of dilute sulphuric acid and adding an equivalent amount of barium hydroxide solution. On evaporation *in vacuo* over sulphuric acid, the guanidine is obtained in deliquescent crystals.

Guanidine is a strongly alkaline, crystalline substance having a caustic taste. It is readily soluble in water and alcohol. On exposure to air it deliquesces and absorbs carbon dioxide with conversion into the carbonate.

When boiled with baryta water, guanidine yields ammonia and urea, thus: $NH:C(NH_2)_2 + H_2O = NH_3 + CO(NH_2)_2$. The urea further splits up into ammonia and carbon dioxide. With hot concentrated acids and alkalies these are the sole products.

Guanidine is a monovalent base which forms a series of crystallisable salts with acids. *Guanidine nitrate*, CH_5N_3,HNO_3, forms crystalline plates which are sparingly soluble in water. The *platinichloride* B_2,H_2PtCl_6 is sparingly soluble in absolute alcohol.

Many salts of guanidine, including the nitrate, sulphate, carbonate, and hydrochloride, give with Nessler's reagent a white or faintly yellowish precipitate, at first flocculent and bulky, but collecting together after a time. E. Schulze (*Ber.*, 1892, **25,** 661) describes the reaction as very delicate; a 0.05 % solution giving an appreciable precipitate, while even a 0.01 % solution is rendered turbid.

Guanidine is not precipitated by lead acetate, but is separated very completely by phosphotungstic acid, which reagent is employed by E. Schulze for its isolation from plant-substances.[1]

Guanidine may be conveniently purified and estimated by precipitating the solution of one of its salts by an aqueous solution of picric acid. Guanidine picrate requires 2600 parts of cold water for solution, and is only sparingly soluble in alcohol and ether. It crystallises in very characteristic forms, does not melt at 280°, but burns at a higher temperature. It does not detonate when struck.

When equivalent weights of guanidine and phenol are dissolved in

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[1] Vetch-seedlings which had grown for 3 weeks in the dark were dried, powdered, and digested with rectified spirit. The extract was filtered, distilled, the residue treated with water and some tannin, and then precipitated by lead acetate. The filtered liquid was precipitated with phosphotungstic acid, the precipitate washed with dilute sulphuric acid, and decomposed with cold lime-water. The filtered liquid was freed from lime by carbon dioxide, the filtrate neutralised with nitric acid, and concentrated on the water-bath. On cooling, guanidine nitrate crystallised out, 1 grm. being obtained from 3 kilogrm. of vetch-seedlings.

hot alcohol, triphenyl-guanidine is formed, and on adding picric acid to a solution of this compound, the corresponding picrate, $CH_2Ph_3N_3,-C_6H_2(NO_2)_3OH$, is obtained as a precipitate of slender needles, m. p. 208°, and requiring about 12,000 parts of cold water for solution. Guanidine *aurichloride*, $CH_5N_3,HAuCl_4$, forms long yellow needles difficultly soluble in water.

Guanidine forms an insoluble compound with mercuric oxide, which fact is utilised by Gergers and Baumann (*Pflüger's Archiv.*, **12**, 205) for the isolation of the base from urine.[1]

Guanidine has markedly poisonous properties. In dogs it produces paralysis, convulsions, vomiting, and difficult breathing; in frogs, muscular twitchings, paralysis, and (with a dose of 0.050 grm.) death.

Methyl-guanidine, $NH:C(NH_2).NH(CH_3)$, is produced by boiling creatine with mercuric oxide, or with lead dioxide and dilute sulphuric acid. It has been isolated by Brieger from putrefying horse-flesh, and has been found in other decomposing animal matters.[2]

Methylguanidine forms a strongly alkaline, deliquescent, crystalline mass, which evolves ammonia and methylamine when boiled with alkali hydroxide. B,HCl crystallises in needles insoluble in alcohol. $B,HAuCl_4$ forms rhombic crystals, melting at 198°, very sparingly soluble in cold water, but readily dissolved by alcohol or ether. Methyl-guanidine picrate, when first precipitated, forms a resinous mass, which, by boiling with water, is converted into needles melting at 192° and soluble in boiling absolute alcohol.

Methylguanidine (with dimethyl-guanidine) occurs in normal urine. For their isolation a large amount of urine is filtered through infusorial earth, the filtrate precipitated with phosphotungstic acid in the usual

[1] The urine is precipitated with barium hydroxide solution, the filtrate neutralised by hydrochloric acid and evaporated to a syrup at 100°. The residue is exhausted with alcohol, the filtered liquid evaporated, and the residue taken up by a little water. The solution is shaken with freshly precipitated mercuric oxide, and allowed to stand for two days in a warm place, when the precipitate is filtered off, treated with hydrochloric acid, and the mercury precipitated by hydrogen sulphide. The filtered liquid is evaporated and the residue dissolved in absolute alcohol. The solution is treated with platinic chloride, the precipitate of ammonium platinichloride removed by filtration, and the filtrate evaporated to a small bulk, when on long standing guanidine platinichloride crystallises out.

[2] Bocklisch isolated methyl-guanidine from impure cultures of *Vibrio proteus* in beef-broth. The ptomaïnes were precipitated by mercuric chloride in the manner directed by Brieger and the precipitate decomposed by hydrogen sulphide in the usual way. The filtrate from the mercuric sulphide was concentrated and treated with a solution of sodium picrate, which precipitated the methyl-guanidine together with creatinine and cadaverine. On boiling the precipitate with absolute alcohol, the cadaverine picrate remained undissolved. The filtrate was evaporated, and the residue taken up with water, the solution acidified with hydrochloric acid and shaken with ether to remove the picric acid, and the aqueous layer treated with auric chloride, when the gold salt of methylguanidine was precipitated, that of creatinine remaining in solution.

manner and the precipitate, containing these bases, washed with
dilute sulphuric acid. The precipitate is then freed from sulphuric
acid and phosphotungstic by cold precipitation with barium hydroxide,
filtered, the precipitate well washed and the wash water added to the
filtrate, the barium removed by carbon dioxide, concentrated and
filtered. The solution is then faintly acidified with nitric acid, a
solution of 20% silver nitrate added so long as a visible precipitate
forms, and the precipitate removed. To the filtrate is then added
further silver nitrate in small portions, until a drop of the fluid when
dropped into a saturated solution of barium hydroxide gives a brown
precipitate. A cold saturated solution of barium hydroxide is then
added, in small portions, until a drop of the clear supernatant fluid
when tested with a solution of silver nitrate in ammonia (10%
silver nitrate, to which ammonia has been added until the precipitate
has just gone into solution, then another drop of NH_3) yields no white
precipitate. The precipitate is then filtered, and to the filtrate barium
hydroxide is added again in small portions so long as a precipitate forms,
an excess being avoided. In this precipitate are the methyl-guanidines.
The precipitate should be collected, washed with barium hydroxide
solution, suspended in water, acidified with sulphuric acid and the silver
removed with hydrogen sulphide, filtered hot, and the filtrate concen-
trated on the water-bath. The sulphuric acid is then carefully removed
with baryta water, traces of excessive barium removed with carbon
dioxide, and the solution again concentrated. A small portion of the
final fluid is then tested with an aqueous solution of picrolonic acid. If
a precipitate occurs, the whole material is to be precipitated. If not,
then the precipitation with silver is to be repeated, as described, and
the final solution again tested with picrolonic acid. If no precipitate
occurs the second time, it may be inferred that the methyl-guanidines
are not present. If a precipitate be secured, it is to be collected,
washed with a small amount of cold water, then dissolved in hot water,
concentrated and recrystallised. The crystals are needles that melt
with foaming at 291°, though if the dimethyl-guanidine be present,
this will not be obtained. There is no known way to separate the
dimethyl-guanidine. The methyl-guanidine forms a thiocyanate that
melts at 175°, with the formation of a red mass. *Benzoyl-methyl-guani-
dine* is a very insoluble salt, that melts at 184°. The double salts
of the bases and gold or platinum are also sharply defined compounds.

Vitiatine is another methyl-guanidine derivative supposed to be

$$\begin{array}{ccc} \diagup N(CH_3)-CH_2-CH_2-HN\diagdown & \\ C=NH & HN=C \\ \diagdown NH_2 & H_2N\diagup \end{array}$$
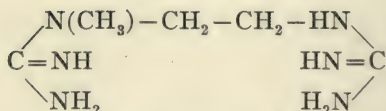
that has been isolated from human urine and from extract of beef. It forms an aurichloride, m. p. 167°.

Methyl-guanidine has marked poisonous properties, the symptoms observed being rapid respiration, mydriasis, paralysis, convulsions, and death. Brieger found it to produce choleraic symptoms.

Creatine. Methyl-glycocyamine. Methyl-guanidine-acetic acid.

$$\begin{array}{c} \diagup NH_2 \\ C=NH \\ \diagdown N(CH_3).CH_2.COOH \end{array}$$
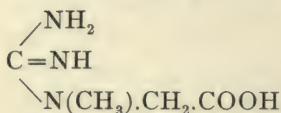
Creatine has been obtained synthetically by heating sarcosine with an alcoholic solution of cyanamide, thus:

$$CH_2NHMe.COOH + CN.NH_2 = NH:C(NH_2).(NMe).CH_2.COOH.$$
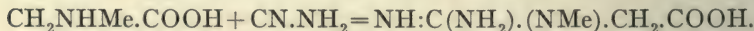
Creatine is a constant constituent of muscle-substance, the flesh of fowls being said to contain 0.32%, cod-fish 0.17, and beef 0.07%. But its isolation from these would not prove its pre-existence, since it is very readily formed by the hydration of creatinine, into which substance, on the other hand, creatine is very easily changed. Creatine exists preformed.

Creatine is most conveniently prepared from Liebig's extract of meat,[1] which sometimes contains granular crystals of the base. The extract should be dissolved in about 20 parts of water and the solution precipitated by a slight excess of basic lead acetate. The filtered liquid is treated with hydrogen sulphide, again filtered, and concentrated to a syrup at a moderate temperature avoiding ebullition. Creatine crystallises out on standing in a cool place for some days. A more complete precipitation is effected if 2 or 3 volumes of alcohol be added. The precipitate of creatine is collected on a filter, washed with rectified spirit, and recrystallised from water.

Creatine is a white opaque substance, but it crystallises with one molecule of water in colourless, transparent rhombic prisms (Fig. 15), which, when heated to 100°, lose their water and become opaque.

[1] Creatine was first isolated by Chevreul, in 1835, from a commercial meat extract of bouillon on which he was requested to report.

Creatine is soluble in 75 parts of cold, and very soluble in hot, water. It is only very slightly soluble in absolute alcohol, more soluble in dilute spirit, and insoluble in ether.

On cooling a strong aqueous solution of creatine, the base separates in bulky needles. On more gradual evaporation of a dilute solution it is deposited in large prisms.

The aqueous solution of creatine has a slightly bitter taste, and is neutral to litmus. With acids, creatine reacts as a monacid base, and combines to form crystallisable salts. The sulphate forms slender prisms, and the nitrate and hydrochloride thick short prisms. Creatine also unites with various neutral salts to form crystallisable compounds. That with zinc chloride forms small crystals, decomposed by water into its constituents. Creatine is not precipitated by lead acetate or phospho-tungstic acid. When oxidised with permanganate, methyl-guanidine is formed. Oxidation with hydrogen peroxide in the presence of a little ferrous sulphide yields glyoxylic and formic acids, formaldehyde

Fig. 15.—Creatine (after Frey).

and methyl-guanidine. It does not combine additively with formaldehyde, as do the amino-acids. When oxidised with alkali, amines are formed.

Conversion of creatine into creatinine, with loss of the elements of water, takes place when solutions of creatine salts are heated. Also, on passing a current of hydrogen chloride over solid creatine, it is converted into creatinine hydrochloride. On evaporating a solution of creatine with the calculated quantity of dilute sulphuric acid, it yields creatinine sulphate. Conversion also occurs very readily on boiling creatine with dilute hydrochloric acid, and the resultant creatinine can be readily identified by conversion into the zinc chloride compound. Creatine is also completely converted into creatinine by heating for 10 hours with dilute acetic acid. Only when effected under certain circumstances is this conversion into creatinine quantitative. It is certainly accomplished by heating for 30 minutes with normal hydrochloric acid in an autoclave at 117°.

When boiled with aqueous barium hydroxide, creatine is decomposed into urea and sarcosine, methyl-hydantoin being also formed. Sarcosine and urea are also formed when creatine is heated in a sealed tube to

150° with an alkaline solution of barium chloride. The urea is further decomposed by the treatment, so that $2NH_3 + CO_2$ are formed from one molecule of creatine. By heating with phosphoric acid to 150°, creatine yields methyl-hydantoin and one molecule of ammonia, whereas urea yields $2NH_3$ under similar treatment. On heating an aqueous solution of creatine with mercuric oxide, oxalic acid and methyl-guanidine are formed:

$$C_4H_9O_2N_3 + 2HgO = C_2H_2O_4 + C_2H_7N_3 + 2Hg.$$
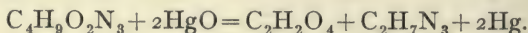
If to 2 c.c. of a cold saturated solution of creatine, 5 or 6 drops of a 20 % solution of silver nitrate be added, and then sufficient solution of potassium hydroxide to dissolve the precipitate first formed, the liquid soon sets to a transparent jelly, and on heating separation of metallic silver takes place.

Creatine does not precipitate a solution of zinc chloride, a behaviour which distinguishes it from creatinine.

Creatine is also distinguished from creatinine by being unprecipitated by a solution of phospho-tungstic acid in presence of hydrochloric acid.

Creatine reduces Fehling's copper solution on long boiling, but no separation of cuprous oxide takes place. When heated with soda-lime, creatine yields methylamine.

Creatine may be estimated in the following manner: In one sample of the urine or other material, an estimation of the creatinine is made according to the method of Folin (p. 316). Another sample is then heated for a half hour in an autoclave at 117° with N-hydrochloric acid, following which another estimation of creatinine is made. The difference represents the creatinine formed from creatine, and may be determined by direct calculation. If the solution contains sugar, this method is not applicable, on account of the colour due to the action of the acid upon the sugar. Rose has shown that by the use of phosphoric acid (half-normal) the conversion of creatine into creatinine may be accomplished and estimated in the presence of sugar.

Creatine occurs normally in the urine of infants and children. It occurs also under all conditions in which the body either has no sugar to burn or is unable to burn it; in starvation, diabetes, sepsis. It occurs also under circumstances of exaggerated wasting of muscle substance. On ingestion, creatinine is eliminated in part unchanged if the dose be large; otherwise it is in small part eliminated as creatinine, in greater part burned, or decomposed in the intestine. On admin-

istration of glycocyamine, creatine appears in the urine. Though it is believed that the creatinine of the urine is derived from creatinine of the muscle, the conditions of transformation are not understood.

Creatinine. Methyl-glycocyamidine. Methyl-guanidine-acetic acid anhydride.

$$C_4H_7ON_3; \ i.e.,$$

$$\begin{array}{c} NH \overline{} CO \\ \diagup \qquad \qquad | \\ C = NH \qquad | \\ \diagdown \qquad \qquad | \\ N(CH_3) - CH_2 \end{array}$$
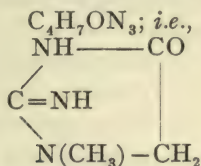
Creatinine is an anhydride of creatine, $C_4H_9O_2N_3$, and is produced from the latter substance with great facility. Creatinine occurs constantly in normal human urine, the amount varying from 1.4 to 2.2 grm. per diem. Creatinine has been found in sweat and in the muscles of fishes, but apparently does not exist preformed in mammalian muscles.

Creatinine may be isolated from human urine by Liebig's process, which consists in exactly neutralising the liquid with milk of lime, and adding calcium chloride as long as calcium phosphate continues to be precipitated. The filtrate, which should be neutral or very faintly acid, is evaporated to a small bulk, and the crystals of common salt, etc., removed. 32 parts of the mother-liquor are treated with 1 part of zinc chloride in very concentrated solution, and the whole left for several days. The creatinine-zinc chloride, which separates in nodules, is washed with a little cold water, and then with alcohol. It is then boiled with recently precipitated lead hydroxide, the filtrate evaporated, and the residue digested with absolute alcohol, which dissolves the creatinine, leaving any creatine insoluble.

There are two fractions of creatinine in the urine of man—an exogenous fraction, due to the ingestion of creatinine-bearing flesh; and an endogenous fraction, derived from the metabolism of the muscular system. The endogenous creatine varies from 1.25 to 2.22 grm. per day, depending upon the amount of muscular tissue in the individual, of which it represents the coefficient. It is an independent metabolism, and the endogenous creatinine is not dependent upon the total protein of the diet—it is a constant in each individual. It is not increased by muscular exercise, unless excessive or done in the untrained subject.

Because of lack of control of the endogenous creatinine, most of the reported observations bearing on the pathological variations in the urinary creatinine are not to be relied upon. In the acute stage of muscular dystrophies and degenerations, and in some fevers, the creatinine is increased. Late in the muscular dystrophies, it may be decreased. When creatine appears, the creatinine is correspondingly diminished except in starvation.

As ordinarily obtained from urine, creatinine crystallises in oblique rhombic prisms and stellate forms (Fig. 16). It dissolves in about 11 parts of cold water, and is sparingly soluble in alcohol, but insoluble in ether.

The aqueous solution of creatinine is neutral. The solution readily undergoes change with formation of creatine, especially if ammonia, oxide of lead, or other base be present. By prolonged boiling with alkali hydroxide, creatinine is completely decomposed.
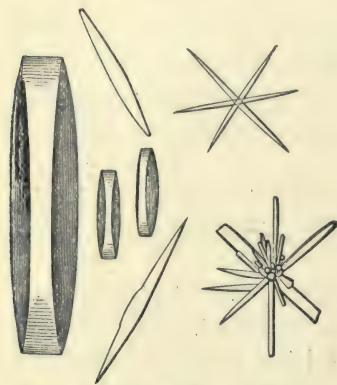
FIG. 16.—Creatinine (after Frey).

By boiling with baryta water, creatinine is hydrolysed to ammonia and methyl-hydantoin:

$$C(NH) \left\{ \begin{array}{l} N(CH_3).CH_2 \\ NH \!-\!\!-\! CO \end{array} \right\} + H_2O = NH_3 + CO \left\{ \begin{array}{l} N(CH_3).CH_2 \\ NH \!-\!\!-\! CO \end{array} \right.$$
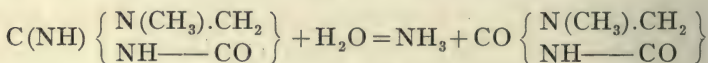
Boiled with water and mercuric oxide, it gives methylguanidine and oxalic acid (compare creatine). Heated with an alkaline solution of barium chloride, under pressure to 150°, creatinine behaves like creatine, but is only partially decomposed by phosphoric anhydride at the same temperature.

Creatinine yields a series of crystallisable salts. The *hydrochloride*, B,HCl, crystallises in short transparent prisms from alcohol or in large laminæ from water. It unites with zinc chloride to form the double salt $ZnCl_2,2BHCl$. This is very soluble in water and alcohol, and must not be mistaken for the compound $ZnCl_2,2C_4H_7ON_3$, which is one of the most characteristic salts of creatinine. *Creatinine-zinc chloride* is obtained by mixing concentrated aqueous or alcoholic solutions of zinc chloride and creatinine, or by adding sodium acetate

to the solution of the double hydrochloride. It forms oblique rhombic prisms or small needles, which have a tendency to form rosettes or warty concretions. The crystals are soluble in about 54 parts of cold or 27 of boiling water. They are insoluble in absolute alcohol, and require 9217 parts of alcohol of 98%, or 5734 of alcohol of 87% for their solution.

Mercuric chloride gives a white, curdy precipitate in strong solutions of creatinine, but the separation is not perfect unless sodium acetate be added, or mercuric acetate substituted for mercuric chloride. On allowing such a mixture to stand at the ordinary temperature, the compound is gradually deposited in microscopic spherules. The compound is almost insoluble in cold water, and is decomposed with partial reduction of the mercury by hot water. It is readily soluble in dilute hydrochloric acid, but is nearly insoluble in acetic acid.

From a concentrated solution of creatinine, silver nitrate precipitates crystals of the compound, $C_4H_7ON_3,AgNO_3$. Mercuric nitrate does not precipitate a dilute solution of creatinine till excess of sodium carbonate is added, when $B_2,Hg(NO_3)_2,HgO$ is thrown down as a crystalline precipitate. Creatinine forms double salts with the chlorides of gold and platinum, and also a double salt with potassium picrate.

Creatinine possesses marked reducing properties. The mercury of the spherical salt above described is at once reduced, even in the cold, to the mercurous state and partly to metal on adding alkali hydroxide; contact with boiling water produces a similar change.

Creatinine reduces Fehling's solution on boiling, the blue liquid changing to yellow, but no cuprous oxide separates. Creatinine appears also to prevent the separation of a precipitate when dextrose is present, and hence exerts an interfering action on the application of Fehling's solution to the detection of dextrose in urine. Pavy's solution is reduced by creatinine without precipitation, and may be used for its estimation. Gold and silver are also reduced, but bismuth is not reduced in alkaline solution, a point of importance in the detection of sugar in the urine with the bismuth test.

Phospho-molybdic and phospho-tungstic acids produce micro-crystalline precipitates in solutions of creatinine acidified with nitric or hydrochloric acid. By treating the precipitates with barium hydroxide, free creatinine is obtained.

If a concentrated solution of picric acid be added to normal human urine a small crystalline sediment is gradually formed. On separating

this, and treating it with hot water, uric acid remains, while the greater part dissolves. The soluble portion is a double picrate of potassium and creatinine, which forms lemon-yellow needles or thin prisms, readily soluble in hot water, sparingly in cold alcohol, and almost insoluble in ether. With dog's urine the precipitate produced by picric acid contains little or no uric acid, and the kynurenic acid present is not precipitated.

When a solution of picric acid is added to a solution of creatinine not more dilute than 1 in 3000, on adding a drop of dilute alkali hydroxide a blood-red colour is produced, which is intensified by boiling the liquid. By this reaction the presence of creatinine can be recognised in the urine of man, dog, and rabbit. Acetone gives a similar but less intense colour. Dextrose gives a similar reaction on heating. It is evident that the behaviour of creatinine with picric acid gravely affects the value of that reagent as a test for small quantities of sugar in urine.

T. Weyl (*Ber.*, 1878, 228) has pointed out that if a few drops of very dilute solution of sodium nitroprusside be added to a solution of creatinine, and dilute sodium hydroxide then added drop by drop, a fine ruby-red colour will be produced, which in a few minutes changes to an intense straw-yellow. If the liquid be now acidified with acetic acid and warmed, it turns greenish and prussian blue separates. Guareschi recommends that 10 % solutions of nitroprusside and sodium hydroxide should be used. The reaction is best obtained by first adding sodium hydroxide, and then a few drops of a concentrated solution of the nitroprusside. The reaction is very delicate, and can be obtained with a solution containing 0.03 % of pure creatinine, or with urine containing 0.066 %. In applying the test to urine the absence of acetone should be insured by distilling off a portion, since that substance gives a ruby-red colour with Weyl's test, though no blue colour can be obtained on acidifying, acetic acid merely restoring the yellow colour to red. According to Guareschi, a red colour is also yielded by hydantoin, methyl-hydantoin, and other compounds containing the group $N.CH_2.CO.N$. Creatine gives no reaction with Weyl's test unless the liquid be first boiled with a dilute acid, so as to convert it into creatinine. In this manner, Weyl demonstrated the presence of creatine in milk (*Ber.*, 1878, 11, 2175). Formaldehyde, benzoyl chloride, permanganate, hydrogen peroxide and strong acids act upon creatinine just as they do upon creatine. In ammonia

creatinine is quite soluble, creatine, however, quite insoluble. When creatinine is heated in weak alkaline solution for an hour, it is converted into creatine, though the reaction cannot be relied upon for quantitative estimation.

The estimation of creatinine in urine was previously based on its isolation as creatinine-zinc chloride, which process is preferred by Neubauer. E. Salkowski directs that 240 c.c. of the urine should be rendered alkaline by the cautious addition of milk of lime, and precipitated by calcium chloride. The volume is made up to 300 c.c., and the liquid filtered after ten minutes. 250 c.c. of the filtrate, representing 200 of urine, which must be feebly alkaline, is evaporated to about 20 c.c., and an equal volume of absolute alcohol added. This is subsequently diluted to 100 c.c. with alcohol, allowed to stand 24 hours, and filtered. To 80 c.c. of the filtrate, slightly acidified with acetic acid, zinc chloride is added, and the precipitate collected after 24 hours. The purity of the creatinine-zinc chloride should be proved by a microscopic examination, with a high power, to make certain of its freedom from sodium chloride. It should be completely soluble in hot water (compare page 313).

Instead of weighing the compound of creatinine with zinc chloride, the contained creatinine may be deduced from the amount of ammonia produced on decomposing it with boiling concentrated sulphuric acid. For this purpose the precipitate should be dissolved in the minimum quantity of sulphuric acid, previously diluted with an equal volume of water, and the solution treated as in Kjeldahl's process.

To isolate creatinine from urine or other materials, the method of Folin is to be preferred (*Zeitschr. physiol. Chem.*, 1904, **44**, 223-242). 20 grm. of picric acid dissolved in hot alcohol are added to each litre of urine, and the mixture vigorously mixed. After an hour, the supernatant fluid is decanted, the solid collected upon a Buchner funnel and washed with a saturated solution of picric acid. The moist precipitate is then roughly weighed, to the precipitate from each litre of urine about 40 c.c. of water are added and then half its weight of potassium bicarbonate, and well rubbed up. By the action of potassium hydrogen carbonate the combination with the picric acid is split, without conversion of the creatinine into creatine. The mass is then placed upon a Buchner funnel, filtered, and washed with saturated solution of potassium hydrogen carbonate. The total filtrate is then neutralised with sulphuric acid, 2 volumes of alcohol added and a small amount of

animal charcoal added to decolourise. The potassium sulphate, charcoal and other insoluble matters are then removed by filtration, and the filtrate stood aside for a day, and may be then refiltered, if further precipitation has occurred.

To this alcoholic filtrate containing the creatinine, zinc chloride in saturated alcoholic solution is then added so long as a precipitate forms, and the mixture set aside until the following day. The creatinine salt is then collected upon a filter and washed with 60% alcohol. The precipitate is then suspended in water, lead hydroxide added in excess, the mixture heated to boiling, and then hydrogen sulphide very carefully added so long as soluble lead in the supernatant fluid is precipitated. The mixture is then filtered, the slight excess of lead in the filtrate completely removed with hydrogen sulphide, and the solution concentrated at acid reaction (about $N/2$ acidity) to a small volume on a water-bath. During this concentration, creatine is being converted into creatinine. The sulphuric acid is then removed with barium hydroxide, filtered, and the filtrate concentrated until crystallisation of creatinine begins. After standing for several days, the crystalline creatinine is collected, washed with alcohol and recrystallised from hot water-alcohol.

The quantitative estimation of creatinine is now easily and rapidly accomplished by the Folin method. It is a colourimetric method, based upon the resemblance of the colour of the reaction with picric acid in alkaline solution to that of a standard solution of potassium dichromate. The Duboscq colourimeter is well adapted to the method. A $N/2$ solution of potassium dichromate is the control solution, and it is placed in the one tube of the instrument, and the column set in height at exactly 8 mm. 10 c.c. urine are placed in a 500 c.c. flask, 15 c.c. of a saturated solution of picric acid added, then 5 c.c. of 10% sodium hydroxide. After 5 minutes the flask is filled to the mark with distilled water, well shaken and a portion of the mixture placed in the second tube of the colourimeter. The height of the column of the unknown fluid is then set just to correspond in colour with that of the control solution. When 10 mgrm. of creatinine are treated in this way, the colour, when set at 8.1 mm., is equal to that of the control solution. This is the basis of calculation. Several readings should be made, all within 15 minutes, and the average taken. If x be the reading of the unknown solution, then $\dfrac{8.1}{x} \cdot$ 10 gives the number of mgrm.

of creatinine in 10 c.c. urine. If the urine is so weak in creatinine as to make the reading below 7 mm. or so strong as to make the reading over 12 mm., it is best to make a second test, using the amount of urine that would contain approximately 10 mgrm. of creatinine, and with this make the definitive reading. Warm solutions should be avoided. Sugar, betahydroxy-butyric acid, diacetic acid and acetone make the colour readings unreliable, and must be removed, whenever possible, without affecting the creatinine.

Xanthocreatinine, $C_5H_{10}ON_4$, is the most abundant of the bases isolated by Gautier from muscle by treatment with absolute alcohol. It closely resembles creatinine, from which it differs by CH_3N. Xanthocreatinine forms sulphur-yellow spangles having a slightly bitter taste. On warming, it evolves an odour resembling acetamide. It is very soluble in water, and also dissolves in boiling absolute alcohol. When taken internally it causes sleep, diarrhœa, and vomiting. It turns blue litmus paper red, but appreciably changes red litmus to blue. Xanthocreatinine forms a hydrochloride crystallising in feathery needles. It also yields crystalline compounds with mercuric and zinc chlorides. The platinum salt is very soluble, and crystallises in long sheaves. The gold salt crystallises with difficulty. Xanthocreatinine gives a precipitate with phospho-molybdic acid, and with silver nitrate a flocculent precipitate soluble in hot water, and crystallising therefrom in needles. Xanthocreatinine is not precipitated by nitric or oxalic acid, nor by cupric acetate; this last reaction distinguishing it from the bases of the xanthine-group.

Chrysocreatinine or Crusocreatinine, $C_5H_8ON_4$, resembles creatinine, from which it differs by CHN. It forms beautiful orange-yellow crystals, sparingly soluble in water. The reaction of the solution is strongly alkaline. The base forms a non-deliquescent hydrochloride and a soluble crystalline platinichloride. The aurichloride forms slightly soluble crystalline grains. Chrysocreatinine is not precipitated by nitric or oxalic acid, or by cupric acetate even on boiling. It precipitates alumina from a solution of alum.

Amphicreatinine, $C_9H_{19}O_4N_7$, only exists in small quantity in muscle. It is a feeble base, crystallising in pale yellow prisms, which, on heating to 100°, become opaque, but do not change their form. It has a bitter taste, and is only slightly soluble in cold water. It forms a crystalline non-deliquescent hydrochloride, a platinum salt crystallising in lozenge-shaped plates, soluble in water but insoluble in

alcohol. The gold salt forms hexahedra and tetrahedra, very soluble in water. Amphicreatinine is not precipitated by mercuric chloride or cupric acetate, and gives no reaction with the murexide test.

The following table exhibits in a condensed form the method employed by Gautier for the extraction of the bases above described. It is evident from the process employed that their oxalates are soluble in absolute alcohol.

Fresh meat, finely minced, is digested with tepid water containing in each litre 0.25 grm. of oxalic acid, and 2 c.c. hydrogen peroxide. At the end of 24 hours the liquid is strained off, the last portions being recovered by pressure, the liquid boiled to coagulate albumin, and again filtered, when the filtrate is treated in the following manner:

The liquid is evaporated to dryness at 50°, preferably under reduced pressure, and the residue extracted with cold absolute alcohol. The alcoholic solution is evaporated to dryness and then extracted with hot absolute alcohol, the liquid decanted and allowed to stand. Ether (sp. gr., 0.725) is added to complete precipitation. The solution is allowed to stand, when after a time (varying with the purity of the ether used) a mass of crystals separates out, which is filtered off and washed with cold absolute alcohol.

A. Alcoholic Washings. Evaporate to dryness, take up residue with water, add slight excess of cupric acetate, and bring to the boiling point. Filter from precipitate, suspend it in hot water, and pass sulphuretted hydrogen through the hot liquid, and filter hot. The filtrate on cooling deposits *pseudoxanthine*.	B. Crystals are treated with boiling absolute alcohol, and the liquid filtered hot.			
	Solution is allowed to become cold and filtered.		Residual crystals are dissolved in hot water, and the liquid allowed to cool, and filtered after standing.	
	Filtrate. Add to alcoholic washings at A.	Crystals consist of *xantho-creatinine* and two unnamed bases of the formula $C_{11}H_{24}O_5N_{10}$ and $C_{12}H_{25}O_5N_{11}$.	Filtrate. Concentrate by evaporation and allow to stand, when *chrysocreatinine* separates out.	Crystals consist of— *amphicreatinine*.

Separation of Flesh Constituents.—The following table, compiled from various sources, gives a systematic method for the separation of the leading constituents of flesh. It may also be applied to extract of meat, urine, and other animal products. It is doubtful if the yield of this method may be assumed to have been preformed.

SEPARATION OF FLESH CONSTITUENTS.

Extract from one-half to one pound of the finely-divided flesh with cold water, repeating the extraction several times. Filter the mixed extracts, boil and filter.

Filtrate. Add basic lead acetate, avoiding excess, allow to stand 48 hours, and filter.

- **Precipitate consists of protein.**

- **Precipitate consists of lead compounds of lead with phosphoric and uric acids, and inositol.**

  Suspend precipitate in warm water, add a few drops of a solution of sodium carbonate, and pass sulphuretted hydrogen gas through the liquid, and filter. Boil the filtrate to expel sulphuretted hydrogen, and add lead acetate, avoiding excess, and filter.

  - **Filtrate** contains the inositol. Remove the excess of lead by means of hydrogen sulphide, and filter. Filtrate will contain *inositol*.

  - **Precipitate.** Decompose with hydrogen sulphide, and filter. Add HCl to filtrate, and allow to stand several days. Filter from any deposit.
    - **Filtrate** contains the *phosphoric acid*.
    - **Deposit** consists of *uric acid*.

- **Filtrate.** Pass hydrogen sulphide gas, filter, and make filtrate up to 250 c.c. Allow to stand several days, and filter from any amorphous or crystalline deposit. Wash the deposit with alcohol.

  - **Filtrate.** Divide into two portions.

    I. Boil off alcohol, and dilute to 150 c.c., make slightly alkaline with ammonia, and add a solution of ammonio-nitrate of silver. Filter and wash precipitate, which consists of compounds of silver nitrate with xanthine, hypoxanthine, and guanine, with dilute ammona. Dissolve the precipitate in boiling nitric acid, sp. gr. 1.1, and decant liquid from any AgCl. Allow solution to stand for six hours, and filter from any crystals that may form.

      - **Crystals.** Treat with ammonio-nitrate of silver (to neutralise any adhering nitric acid), suspend in boiling water, and pass hydrogen sulphide gas through the liquid. Filter and allow filtrate to stand, when crystals of *hypoxanthine* will form.

      - **Filtrate.** Add slight excess of ammonia, and filter off the precipitated xanthine silver compound. Decompose precipitate, suspended in hydrogen sulphide gas, and filter hot. Concentrate filtrate, which on cooling will deposit xanthine and guanine. Treat crystals with dilute ammonia.
        - **Residue.** Dissolve in HCl, and add cold saturated solution of picric acid, when *guanine* picrate will be precipitated.
        - **Filtrate** contains the *xanthine*.

    II. Evaporate to low bulk, and filter from any crystals.

      Crystals consist of leucine, tyrosine, and perhaps creatine. The crystals are treated with hot 70% alcohol and filtered.

      - **Residue of** crystals, insoluble in alcohol, consists of *tyrosine*, which may be purified by crystallisation from boiling water.

      - **Filtrate.** On cooling, *leucine* crystallises out, which may be purified as follows—Boil for half an hour with dilute potassium hydroxide containing a little oxide of lead. The lead sulphide is filtered off, the filtrate exactly neutralised with dilute sulphuric acid, and evaporated to dryness at 100°C. The residue is extracted with boiling alcohol to extract the *leucine*.

      - **Filtrate** is treated with solutions of sodium acetate and syrupy zinc chloride, when *creatinine* will be precipitated.

  - **Deposit** consists of *creatine*.

PURINE BASES. PYRIMIDINE BASES.

The purine bases are members of a most important group of substances concerned in both plant and animal physiology. Although their rôle in plant physiology is not so clear, in animal physiology the purines may be in general defined as the end-products of the metabolism of nucleic acid, of the nuclei of the cells. There is a regular wear-and-tear of cells in daily life, and cells die and are replaced by new cells. The nuclei of such degenerated and replaced cells undergo autolysis in the tissues in which they were resident, this being a reaction of digestion, a hydrolysis that splits the nuclein into protein and nucleic acid, and the latter into its three components, phosphoric acid, purine and pyrimidine bases and pentose. It is the purine derived from this nucleic acid that constitutes the larger source of the purine bases of the urine. In the muscles a purine, hypoxanthine, is combined with a pentose in the protoplasm of the muscle cells outside the nuclei, being the only known extra-nucleic purine in the higher animal body, and from this a small fraction of hypoxanthine is daily derived. The purine metabolism is independent of the metabolism of common protein in the higher animals; in the birds and some reptiles, the purines are the end-products of the common protein metabolism. But in the human metabolism, the purines are not so related.

The purine bases are members of the group of purines, derivatives from the purine nucleus. This is to be regarded as a closed ring, and the different elements have for convenience of nomenclature been numbered.

$$
\begin{array}{ccc}
N_1 & \!\!\!\!-\!\!\!\! & C_6 \\
| & & | \\
C_2 & & C_5 \!\!-\!\! N_7 \\
| & & | \qquad \diagdown \\
& & \qquad\qquad C_8 \\
| & & | \qquad \diagup \\
N_3 & \!\!-\!\! & C_4 \!\!-\!\! N_9
\end{array}
$$
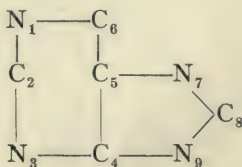
The structures of the purine and the derivatives that occur in the animal body as the expressions of its endogenous metabolism are as follows:

Purine.
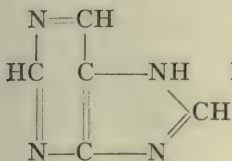
$$N = CH$$
$$HC \quad C — NH$$
$$\qquad\qquad\qquad >CH$$
$$N — C — N$$

Adenine.
6-amino-purine.

$$N = C.NH_2$$
$$HC \quad C — NH$$
$$\qquad\qquad\qquad >CH$$
$$N — C — N$$

Guanine.
2-amino-6-oxypurine.

$$NH — CO$$
$$H_2N.C \quad C — NH$$
$$\qquad\qquad\qquad >CH$$
$$N — C — N$$

Hypoxanthine.
6-oxypurine.

$$HN — CO$$
$$HC \quad C — NH$$
$$\qquad\qquad\qquad >CH$$
$$N — C — N$$
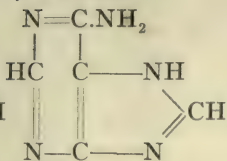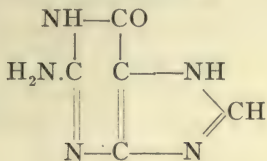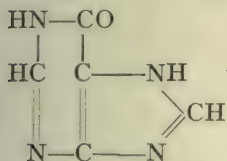
Xanthine.
2, 6-dioxypurine.

$$HN — CO$$
$$OC \quad C — NH$$
$$\qquad\qquad\qquad >CH$$
$$HN — C — N$$

Uric acid.
2, 6, 8-trioxypurine.

$$HN — CO$$
$$OC \quad C — NH$$
$$\qquad\qquad\qquad >CO$$
$$HN — C — NH$$
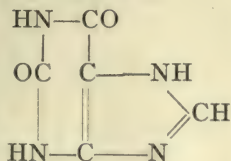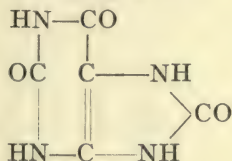
Two bases, termed episarkine and carnine, have been reported present in the urine. The descriptions are vague, and their structures have not been elucidated.

In plants we have not only aminopurines, but also a special class, the methylpurines. These are present in many plants, but in particular in tea, coffee and cocoa. As a result therefore of the ingestion of these beverages, the methylpurines are introduced into the human body. The body is able to demethylate these bases in part, but never completely, i.e., at least one methyl group is always left to distinguish these purines from those derived from the nucleic catabolism. The structures of these methylpurines and of the methylpurines that are eliminated in the urine as the result of the ingestion of these beverages, are as follows (see vol. 6):

Caffeine.
1, 3, 7-trimethyl-2, 6-dioxypurine.

$$CH_3.N — CO$$
$$\qquad\qquad\qquad CH_3$$
$$OC \quad C — N$$
$$\qquad\qquad\qquad >CH$$
$$CH_3.N — C — N$$

Theophylline.
1, 3, dimethyl, 2, 6-dioxypurine.

$$CH_3.N — CO$$
$$OC \quad C — NH$$
$$\qquad\qquad\qquad >CH$$
$$CH_3.N — C — N$$
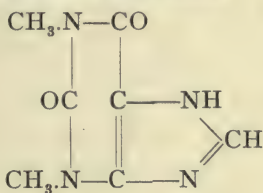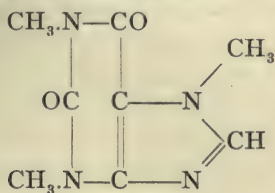
Theobromine.
3, 7 dimethyl-2, 6-dioxypurine.

$$
\begin{array}{c}
\text{HN—CO} \\
\quad\quad\quad \diagup \text{CH}_3 \\
\text{OC}\quad\text{C——N} \\
\quad\quad\quad \diagdown \text{CH} \\
\text{CH}_3\text{.N—C——N}
\end{array}
$$

Methyl-xanthine.
1-methyl-2, 6-dioxypurine.

$$
\begin{array}{c}
\text{CH}_3\text{.N—CO} \\
\text{OC}\quad\text{C——NH} \\
\quad\quad\quad\quad \diagdown \text{CH} \\
\text{HN—C——N}
\end{array}
$$
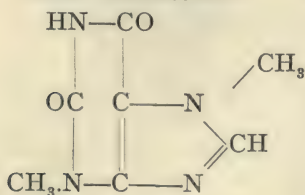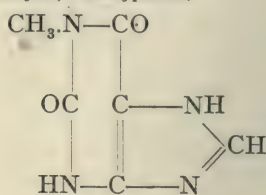
Heteroxanthine.
7-methyl-2, 6-dioxypurine.

$$
\begin{array}{c}
\text{HN—CO} \\
\quad\quad\quad \diagup \text{CH}_3 \\
\text{OC}\quad\text{C——N} \\
\quad\quad\quad \diagdown \text{CH} \\
\text{HN—C——N}
\end{array}
$$
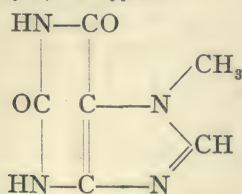
Paraxanthine.
1, 7-dimethyl-2, 6-dioxypurine.

$$
\begin{array}{c}
\text{CH}_3\text{.N—CO} \\
\quad\quad\quad \diagup \text{CH}_3 \\
\text{OC}\quad\text{C——N} \\
\quad\quad\quad \diagdown \text{CH} \\
\text{HN—C——N}
\end{array}
$$

Epiguanine.
7-methyl-2-amino-6-oxypurine.

$$
\begin{array}{c}
\text{HN—CO} \\
\quad\quad\quad \diagup \text{CH}_3 \\
\text{NH}_2\text{.C}\quad\text{C——N} \\
\quad\quad\quad \diagdown \text{CH} \\
\text{N—C——N}
\end{array}
$$
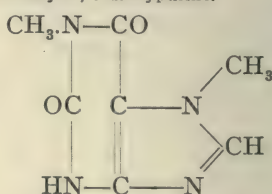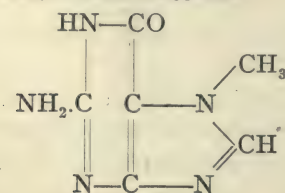
From a contemplation of these structures, it is obvious that amino-purines are converted into oxypurines by deaminization and oxidation, uric acid being the completed stage. The methylpurines are demethylated, but incompletely, one or two methyl groups being always left; complete oxidation to uric acid cannot be accomplished.

In all work and investigations with the purine bases, the exogenous and the endogenous fractions must be carefully separated. A purine-free diet must be employed with all subjects in whom the estimation of the purines is to be attempted. Milk, eggs and rice constitutes a practically purine-free diet. The glandular meats (sweetbreads) are most rich in purines, all flesh contains some, the seed vegetables (peas and beans) contain quite notable amounts; and tea, coffee and cocoa are very rich in them, malted liquors fairly rich also. In the tissues and urine of a subject on a purine-free diet, only uric acid, xanthine, hypoxanthine, adenine and guanine are ever to be found. All the methyl-

purines to be found in the urine are derived from beverages. In mixed urines of individuals on mixed diets, three-quarters of the purine bases are methylpurines derived from beverages. The purine output on a purine-free diet as an index of the nucleic metabolism has no relation to the metabolism of common protein and is also independent of the creatinine metabolism. The total purine nitrogen output per day in health on a purine-free diet will vary from 0.1 to 0.2 grm., of which from 5 to 10 % may be in the form of purine bases.

In their chemical and physical characters, the purine bases present resemblances to uric acid. They are mostly slightly soluble in cold water, and, except caffeine and theobromine, are insoluble in alcohol, ether, or chloroform. They all yield white precipitates with phosphomolybdic acid, mercuric chloride, and ammoniacal lead acetate; and guanine and adenine are very perfectly precipitated by picric acid.

A general reaction of the purine bases (including uric acid) is their precipitation from ammoniacal solutions by ammoniacal silver nitrate, as a gelatinous compound of the base with argentic oxide. The xanthine compound contains $C_5H_4O_2N_4,Ag_2O$. The precipitates are usually insoluble in ammonia, unless concentrated and used in large excess, but to ensure complete precipitation excess should be avoided. On treating the precipitates with dilute nitric acid of 1.10 sp. gr., they are converted into compounds of the bases with silver nitrate, xanthine forming $C_5H_4O_2N_4,AgNO_3$. These compounds are well-defined crystallisable substances, insoluble in water, and, in the cases of hypoxanthine, carnine, adenine and episarkine, insoluble in nitric acid of the above strength, even on boiling; or, at any rate, crystallising out rapidly on cooling. Guanine, carnine, adenine, and episarkine are stated by G. Salomon (*Zeit. physiol. Chem.*, **18,** 207) to behave similarly, but, according to J. L. W. Thudichum, the silver nitrate compound of guanine dissolves tolerably easily in hot dilute nitric acid, and is only very gradually deposited on cooling.[1] The compounds of xanthine, heteroxanthine, and paraxanthine remain in solution after cooling, which difference of behaviour permits of their separation from the bases previously mentioned. The bases are all completely reprecipitated as their silver-oxide compounds on neutralising the nitric acid solution by ammonia. Heteroxanthine

[1] G. Bruhns (*Ber.*, 1890, **23,** 225) utilises the same behaviour for the separation of xanthine and guanine from hypoxanthine and adenine.

and paraxanthine may be separated from xanthine by taking advantage of the limited solubility of their sodium salts in sodium hydroxide and from each other by utilising the sparing solubility of the hydrochloride of heteroxanthine.

Most of the purine bases are precipitated by cupric acetate, especially on heating. A still more perfect separation is effected by cuprous solutions, the precipitate consisting in each case of the cuprous salt of the derivative. These compounds may be obtained by treating the neutral solution with a mixture of cupric sulphate and sodium sulphite or thiosulphate, or by mixing the ammoniacal solution with Fehling's solution, heating to boiling, and gradually adding a solution of dextrose, Instead of cuprous oxide separating in the free state, it combines with the xanthine derivative to form a white insoluble compound. Hence it is evident that the presence of xanthine and its allies, including uric acid, may prevent the detection of sugar in urine by Fehling's test to an extent dependent on the amount of the interfering body present. The fact is of considerable practical importance when small quantities of sugar are to be sought for.

If, instead of using dextrose, the mixture of the alloxur-base with Fehling's solution be treated with hydroxylamine hydrochloride, reduction of the copper to the cuprous state occurs in the strongly alkaline solution and at the ordinary temperature. Treated in this way, *guanine* and *xanthine* give precipitates which are at first white, but rapidly become green by oxidation. *Heteroxanthine* and *paraxanthine* give similar white precipitates. The *uric acid* precipitate, cuprous urate, $Cu_2O,C_5H_4O_3N_4$, is yellowish-white at first, but rapidly becomes greenish. The *carnine* precipitate is liable to be mixed with uncombined cuprous oxide, which colours it yellow. The *adenine* and *hypoxanthine* precipitates are white. *Theobromine* and *caffeine* are the only known xanthine derivatives which do not give precipitates with the above reagent.

M. Krüger (*Zeit. physiol. Chem.*, **18,** 351) finds that, by the employment of cupric sulphate and sodium hydrogen sulphite, all the xanthinoid substances containing a substituted NH-group in the molecule are precipitated very perfectly from warm solutions as cuprous compounds. Theobromine constitutes a remarkable exception to this rule, being, like caffeine, creatine and creatinine, unaffected by Krüger's reagent. In carrying out the method, Krüger slightly acidifies the liquid containing the xanthine bases with sulphuric acid. Sodium

bisulphite is then added, and this is followed by cupric sulphate, when the precipitable bases are thrown down as gelatinous or flocculent white precipitates, which gradually become green or brown. In some cases the solution must be heated to ensure complete precipitation, but in others the reaction occurs perfectly in the cold. The precipitates dissolve readily in mineral acids, but only with difficulty in hot acetic acid. They are not altered by sodium hydroxide, but dissolve in ammonia in presence of air. They are readily decomposed by alkaline sulphides.

Uric acid is completely precipitated by Krüger's reagent, as also are adenine, methyladenine, hypoxanthine, and guanine. Dimethyl-hypoxanthine is not precipitated from warm solutions, but from cold concentrated solutions it separates in fine yellow needles.

If sodium thiosulphate (hyposulphite) be substituted for sodium bisulphite, the behaviour of the xanthinoid substances is somewhat different, apparently owing to the formation of compounds soluble in excess of the thiosulphate. Adenine is completely thrown down in cold solutions on standing, and methyladenine and guanine behave similarly, but hypoxanthine is not precipitated even from moderately strong solutions unless warmed. The following table shows the general behaviour of the xanthinoid compounds with Krüger's reagents:

	Cupric sulphate, and	
	Sodium hydrogen sulphite	Sodium thiosulphate
Uric acid	Precipitated.	Precipitated.
Adenine	Ppted.	Ppted.
Methyl-adenine	Ppted.	Ppted.
Hypoxanthine	Ppted.	Ppted. on warming only.
Guanine	Ppted.	Ppted.
Dimethyl-hypoxanthine	Ppted. only from cold concentrated solutions.	Not ppted.
Theobromine	Not ppted.	Not ppted.
Caffeine	Not ppted.	Ppted.

E Salkowski (abst. *Jour. Chem. Soc.*, 1895, **2,** 538) precipitates 1,000 c.c. of urine by ammoniacal silver nitrate, after removing the phosphates by magnesia-mixture. The silver precipitate is suspended in water, decomposed by hydrogen sulphide, and the filtered liquid evaporated to dryness. The residue is treated with a little water containing from 2 to 3 % of sulphuric acid, which dissolves the xanthine bases, leaving the uric acid practically insoluble. After 24 hours, the liquid is filtered and the purine bases reprecipitated by ammoniacal

silver nitrate, and estimated from the weight of silver in the precipitate. By this process, Salkowski found the amount of bases to be about 8 to 10 % of the uric acid and subject to few variations.

The proportion of purine derivatives (other than uric acid) ordinarily present in *urine* is extremely small, but there is reason to believe that, under circumstances not fully understood, their amount is much increased and may then be or pathological importance. For the actual isolation and separation of the purine bases, 5 to 10 gallons of urine should be treated by instalments of about 1 quart at a time with neutral lead acetate in powder, as long as a precipitate is produced. The liquid is filtered and sodium sulphate added as long as lead sulphate is thrown down, the liquid poured off from the precipitate, sodium bisulphite and copper sulphate added, and the liquid boiled. The precipitate, which contains the purine derivatives as cuprous salts, is filtered off, washed, dissolved in dilute nitric acid, and excess of ammonia added, followed by silver nitrate. The precipitate, consisting of the argentic oxide compounds of purine, is separated, suspended in hot acidified water, and decomposed by hydrogen sulphide, the resultant silver sulphide filtered off, and the filtrate concentrated. If, instead of decomposing the silver precipitate with hydrogen sulphide, it be boiled with nitric acid of 1.10 sp. gr. the silver nitrate compounds of hypoxanthine and adenine will crystallise out immediately on cooling, while those of xanthine, paraxanthine, and heteroxanthine will remain in solution, and may be recovered as the silver oxide compounds by rendering the filtrate ammoniacal.

Balke (*J. pr. Chem.*, [*ii*], **47,** 537) employs the copper process for the isolation of the xanthine bases from flesh, and gives the following details of an experiment: About 800 grm. of finely-minced horse-flesh. from which the nerves had been previously removed as far as possible, was digested with an equal weight of water at 50 to 60° for about an hour with constant agitation. The liquid was pressed through a cloth, and the residue similarly treated with half the quantity of water. The liquors obtained were boiled and filtered trom the coagulated protein. One-half of the filtrate[1] was rendered alkaline by sodium hydroxide, and

[1] The other half of the liquid was treated by Neubauer's process as follows:—The clear filtrate was treated with lead acetate, filtered, and the excess of lead removed by hydrogen sulphide. The filtrate was evaporated to a syrup at 100°, and allowed to stand for several days. The creatine which separated was filtered off with the aid of a mercury-pump, and washed with alcohol. The filtrate and washings were heated on the water-bath, ammonia added, and precipitated with ammonio-nitrate of silver. The precipitate was separated, washed with dilute ammonia, and boiled with dilute nitric acid (sp. gr. 1.10) containing a little urea. The liquid was filtered boiling hot, with the aid of a hot-water funnel, and allowed to stand for four hours, when the hypoxanthine com-

filtered from a small precipitate of phosphates. The filtrate was treated with hydroxylamine hydrochloride, and Fehling's solution then gradually added. The supernatant liquid was decanted from the bulky yellowish-brown precipitate, which was washed by decantation with sodium acetate solution, and then collected on a filter and again washed. The precipitate was next suspended in ammonia, and decomposed by hydrogen sulphide. The liquid was filtered from the copper sulphide, concentrated, made ammoniacal, and treated with lead acetate, by which the lead compounds of xanthine, hypoxanthine, and carnine were completely precipitated, and a filtrate obtained which gave no trace of a precipitate with silver nitrate. On boiling the lead precipitate several times with water the carnine compound was dissolved, and the base was obtained from the solution after treating it with hydrogen sulphide. The weight isolated was 0.069 grm. The portion of the lead precipitate insoluble in boiling water was decomposed by hydrogen sulphide, when, by evaporating the filtrate, a mixture of xanthine and hypoxanthine was obtained, as a yellowish-white mass weighing 0.172 grm. This was treated with ammoniacal silver nitrate, and the bases separated by boiling the precipitate with nitric acid of 1.10 sp. gr.

For the isolation of substances of the xanthine-group from *malt*, Balke boils several kilogrm. of the dry grain with water containing 0.5 % of sulphuric acid. The liquid is strained, concentrated to half its bulk, and at once precipitated with neutral lead acetate in excess. The filtered liquid is treated with hydrogen sulphide, filtered, again heated to dissipate the excess of the precipitant, and treated at 80° with a solution of cupric sulphate. The precipitate, which contains the purine bases, is separated, decomposed by hydrogen sulphide, and the filtrate again treated with cupric sulphate. After again decomposing the precipitate with hydrogen sulphide, the filtered and concentrated liquid is neutralised with ammonia and precipitated with silver nitrate, the precipitated bases being separated as before.

Xanthine. $C_5H_4O_2N_4$. 2-6-Dioxypurine.

The constitutional formula is given on page 321. Xanthine was originally discovered by Marcet (1819) in a urinary calculus, and

pound crystallised out in small needles which were filtered off and converted into the hypo-xanthine silver compound by digestion with ammonia. From the acid mother-liquor xanthine silver oxide was precipitated by adding ammonia. From the silver compounds of xanthine and hypoxanthine the bases were obtained as yellowish-white crystalline masses by passing hydrogen sulphide and evaporating the filtrates.

called "xanthic oxide." It has been found in Jarvis Island guano, occurs as a normal constituent of urine especially during the use of sulphur-baths, and is present in minute quantities in yeast, the muscles of mammals and fishes, the liver, spleen, pancreas, thymus, brain, etc. It occurs also in very small quantities in plants, e.g., in tea, malt-seedlings, lupins, etc. The only natural source from which it can be at all conveniently extracted is meat-extract. It has been produced synthetically by A. Gautier (*J. Chem. Soc.*, **48**, 275) by the reaction of hydrocyanic acid and water in presence of acetic acid, but its best mode of preparation is the decomposition of guanine by nitrous acid: $C_5H_5ON_5 + HNO_2 = C_5H_4O_2N_4 + H_2O + N_2$. A nitro-compound is formed at the same time, which yields xanthine on reduction.[1]

Pure xanthine forms a white powder consisting of microscopic crystals. It acquires a waxy lustre by friction. On heating xanthine, a small portion sublimes unchanged, but by far the greater part chars, with evolution of cyanogen, hydrocyanic acid, carbon dioxide, and ammonia.

For the quantitative estimation of the purine bases in urine or tissue extracts, we employ a double precipitation, using first precipitation with copper, lastly with silver. For the estimation of the bases in the urine, not less than 15 litres of urine suffices, and this is little enough. To the 15 litres of urine are added 300 c.c. of strong acetic acid. The urine should be free from albumin. The urine is brought to simmering, then 300 grm. of sodium acetate and 500 c.c. of a 40% solution of sodium bisulphite added and then quickly about 750 c.c. of a 10% solution of copper sulphate, and the mixture kept simmering. The precipitate gradually turns brownish, and after ten minutes the precipitate is collected on a filter, washed thoroughly with hot water, then suspended in about 1 litre of hot water, thoroughly mixed and the copper precipitated by the addition of alkaline sodium sulphide, additional hydrogen sulphide gas passed through until precipitation of copper is complete, then the mixture is acidified with acetic acid, boiled, and filtered hot. The filtrate should be clear. The collected filtrates are then acidified with hydrochloric acid and concentrated to about 50 c.c. and set aside for the crystallisation of the uric acid. On the following day, the uric acid is filtered off, washed with cold

[1] According to E. Fischer (*Annalen*, **215**, 253) the best plan is to dissolve 10 grm. of guanine in a mixture of 20 grm. of concentrated sulphuric acid with 150 c.c. of water. After boiling, the liquid is cooled to about 80°, and 8 grm. of sodium nitrite added with constant agitation. The yield of xanthine is nearly quantitative, the product is of a pale orange colour, and free from the above mentioned nitro-compound.

water three times and the collected filtrates used for the isolation and estimation of the purine bases.

If the attempt is to be made to separate the different purine bases, the method just described is the best to employ. It is, however, of no avail to make this attempt unless at least 50 litres of urine are worked up, otherwise enough of the bases will not be secured to make the isolation successful.

The solution of bases contains still a little uric acid and this must be removed. The solution is first made alkaline, then acidified with acetic acid, heated to about 80°, and about 100 c.c. of a solution of permanganate added (a hot 0.5 % solution of potassium permanganate is decolourised by the addition of alcohol, freshly prepared) then 10 c.c. acetic acid of 10%, and the mixture allowed to stand for a few moments. In this way the uric acid is removed by oxidation. The solution is then cooled, made strongly alkaline with ammonia, and a 10% silver solution added until the clear supernatant fluid shows an excess of silver when tested with nitric acid. The amount of ammonia must be enough to hold the silver chloride in full solution. The gelatinous precipitate is then collected upon a hardened filter paper, the precipitate washed with dilute ammonia until the wash water is free from chloride, then washed a couple of times with cold distilled water, suspended in water in a flask, magnesia added and the mixture distilled until all fumes of ammonia have passed out. A fraction of this solution is then subjected to the Kjeldahl method for the estimation of nitrogen. The result is simply to be calculated as purine-nitrogen. It is also possible to estimate the silver gravimetrically, or by titration with sulphocyanide.

Xanthine is very sparingly soluble in water, requiring 1400 parts of boiling or 14,000 of cold water for solution. The hot aqueous solution deposits a pellicle on evaporation. Its reaction is neutral. In alcohol and ether xanthine is insoluble.

Xanthine dissolves in dilute acids and in alkalies, and unites with each class of substances to form crystallisable compounds (Fig. 17). It dissolves with facility in sodium or potassium hydroxide, but is precipitated from the solution by adding an acid, even carbonic acid. It is dissolved by warm ammonia (distinction from uric acid), and on cooling crystals of the ammonium salt separate; but on exposure to air, or on evaporating the solution, all the ammonia is lost and free xanthine remains.

$NaC_5H_3O_2N_4 + H_2O$ crystallises in microscopic needles from a solution of xanthine in the smallest possible quantity of sodium hydroxide. Like hydrogen sodium urate, it is decomposed by repeated recrystallisation, and retains its water of crystallisation till heated to about 190°. On boiling xanthine with barium hydroxide solution, the sparingly soluble barium salt separates on cooling.

Xanthine hydrochloride, B,HCl, is deposited in difficultly soluble glistening scales aggregated in nodules. The *sulphate*, $B,H_2SO_4 + H_2O$, forms microscopic glistening tables, which lose the whole of their acid on washing with water. *Xanthine nitrate* forms fine yellow crystals of characteristic microscopic appearance (Fig. 17).

FIG. 17.

XANTHINE NITRATE   XANTHINE HYDROCHLORIDE

(after Kühne).

Mercuric chloride precipitates xanthine from very dilute solutions. A solution of 1 part of xanthine in 30,000 gives a distinct opalescence with mercuric chloride.

Cupric acetate produces no precipitate in a cold solution of xanthine, but on heating a flocculent precipitate of apple-green colour is formed. With cuprous salts, or with Fehling's solution in presence of hydroxylamine hydrochloride (page 324), the compound $Cu_2O,C_5H_4O_2N_4$ is thrown down as a white precipitate, which rapidly turns green from oxidation.

An ammoniacal solution of xanthine gives precipitates with the chlorides of calcium and zinc, and with lead acetate.

An ammoniacal solution of xanthine gives a gelatinous precipitate of $Ag_2O,C_5H_4O_2N_4$ with ammoniacal silver nitrate. Treated with hot dilute nitric acid (sp. gr., 1.10) it dissolves, and the solution, after cooling, very slowly (if at all) deposits crystals of xanthine-silver nitrate, $C_5H_4O_2N_4,AgNO_3$, grouped in a manner resembling wavellite. The same compound separates when a solution of xanthine in a minimum of nitric acid is treated with silver nitrate. Its greater solubility in hot nitric acid of the above strength distinguishes the silver nitrate compound of xanthine from those of hypoxanthine, carnine, adenine (and guanine); while those of (guanine,) hypoxanthine and paraxanthine resemble the xanthine compound in their behaviour.
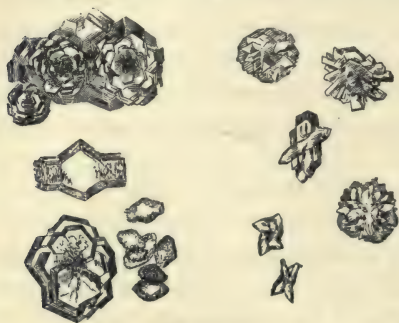
Xanthine gives the following colour-reactions with oxidising agents:

Strecker's Test.—Xanthine dissolves in hot nitric acid without evolution of gas. On careful evaporation of the solution a yellow residue remains, which turns reddish-yellow on addition of potassium or sodium hydroxide, and on subsequent heating becomes reddish-violet. If ammonia be substituted for the fixed alkali in the above test, no violet colouration is obtained. This behaviour distinguishes xanthine from uric acid, which gives the characteristic murexide reaction when similarly treated.

Weidel's Test.—If xanthine be treated with freshly-prepared chlorine-water and a trace of nitric acid, and the liquid carefully evaporated to dryness, a residue is obtained which becomes pink or crimson on cautious exposure to ammoniacal vapours (compare uric acid).

Hoppe-Seyler's Test.—If solid xanthine be sprinkled on a solution of sodium hydroxide with which some bleaching powder has been mixed, each particle becomes surrounded with a dark green ring or scum, which rapidly becomes brown and disappears.

When xanthine is added to an alkaline solution of diazobenzene-sulphonic acid, a red colour develops.

When treated with hydrochloric acid and potassium chlorate, xanthine yields alloxan and urea.

Xanthine occurs in very rare cases as a urinary calculus. For its detection, the powdered calculus should be boiled with alkali hydroxide and the filtered solution treated with hydrochloric acid and again filtered. If xanthine be present in any quantity, hexagonal tables or globular masses of the hydrochloride will form as the liquid cools. The indication may be confirmed by dissolving the product in ammonia and adding ammoniacal silver nitrate, when gelatinous xanthine-silver oxide will be precipitated.

If xanthine be brominated and then methylated, it will be converted into bromo-caffeine, and this is the preparation best adapted to the absolute demonstration of xanthine.

Heteroxanthine, $C_6H_6O_2N_4$, occurs in very small quantity in the urine of subjects consuming tea, coffee or cocoa. It is crystalline, soluble with difficulty in cold water, but much more readily on heating, and is insoluble in alcohol or ether. The crystals melt at 380°. The *hydrochloride* crystallises readily and is only sparingly soluble, which fact gives a means of separating heteroxanthine from the closely-allied base paraxanthine, the hydrochloride of which is more easily soluble.

The two bases may be separated from co-occurring xanthine derivatives by the sparing solubility of their sodium salts in excess of sodium hydroxide.

Heteroxanthine gives an insoluble compound with ammoniacal silver nitrate. It yields a brilliant colour with Weidel's test (page 331), but gives no characteristic reaction with Strecker's test. It does not yield the colour test with diazo-benzene sulphonic acid.

Paraxanthine.—This exists in minute quantity in urine after ingestion of tea, coffee or cocoa. Paraxanthine is sparingly soluble in cold water, but dissolves readily on warming, and is insoluble in alcohol and ether. It crystallises in flat, irregular, hexagonal tables when its solution is slowly evaporated, but if the liquid be rapidly concentrated the base separates in needles.

The silver nitrate compound of paraxanthine is soluble in hot dilute nitric acid, thus resembling the xanthine compound and differentiating paraxanthine from hypoxanthine, carnine, adenine, episarkine, and guanine. Paraxanthine responds to Weidel's reaction, but gives no colour with Strecker's test nor to diazo-benzene-sulphonic acid.

Paraxanthine is further distinguished from xanthine by its greater solubility in water, and from heteroxanthine by the more ready solubility of its hydrochloride.

Guanine. 2-Amino-6-oxypurine. $C_5H_5ON_5$.

Guanine is best prepared from Peruvian guano, which should be boiled with milk of lime till it assumes a greenish-yellow colour, when the liquid is filtered. The operation is repeated as long as a coloured filtrate is obtained. The residue, which contains the whole of the uric acid and guanine, is repeatedly extracted with sodium carbonate. The filtrate is treated with sodium acetate, hydrochloric acid added to strong acid reaction, and the guanine dissolved out of the precipitate by boiling it with dilute hydrochloric acid. The guanine hydrochloride which separates on cooling is separated from admixed uric acid by boiling it with dilute ammonia, and the residual guanine dissolved in hot concentrated nitric acid, which on cooling deposits the nitrate, from which the free base may be liberated by ammonia. C. Wolff (*Zeit. Physiol. Chem.*, **17,** 468) recommends the following method for the preparation of guanine:—Guano is boiled for 4 hours with dilute sulphuric acid, cooled and filtered, the filtrate made alkaline with

sodium hydroxide and again filtered. To the filtrate ammoniacal silver nitrate is added, which precipitates the guanine and uric acid. The washed precipitate is treated with hot dilute hydrochloric acid, the silver chloride filtered off, and the filtrate decolourised with animal charcoal. From the clarified liquid the guanine is precipitated by ammonia. It is redissolved in hot nitric acid containing a small quantity of urea (to ensure the absence of any trace of nitrous acid), and the liquid set aside to crystallise. The guanine nitrate which separates is free from uric acid, and is freed from traces of xanthine by solution in dilute sodium hydroxide and addition of ammonium chloride, when the xanthine remains in solution.

Guanine forms a white amorphous powder which may be heated to 200° without change. It is insoluble in water, alcohol, or ether. Guanine is distinguished from xanthine and hypoxanthine by its insolubility in hot dilute ammonia. It forms crystallisable salts with the stronger acids, and *guanine hydrochloride*, $C_5H_5N_5O,-HCl+H_2O$ and *guanine nitrate*, $2B,HNO_3+3H_2O$, have a characteristic microscopic appearance. The silver nitrate compound of guanine is described on page 330.
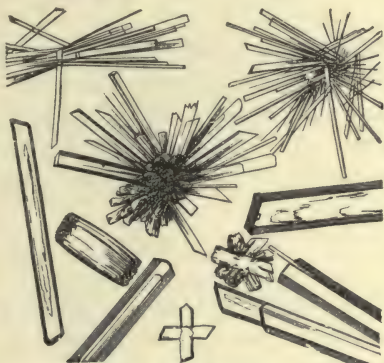
FIG. 18.—GUANINE HYDROCHLORIDE (after Kühne).

On adding a cold saturated aqueous solution of picric acid to a warm solution of guanine hydrochloride, guanine *picrate*, $B,C_6H_2(NO_2)_3OH+H_2O$, is thrown down as a highly insoluble precipitate of orange-yellow silky needles. Adenine is the only other base of the xanthine group which is precipitated by picric acid from dilute solutions, but guanidine gives a similar reaction.

Potassium dichromate throws down from solutions of guanine a highly insoluble, orange-red, crystalline precipitate of guanine dichromate, $B,H_2Cr_2O_7$. Potassium ferricyanide produces a brown, crystalline precipitate. Xanthine and hypoxanthine give no similar reactions. Guanine forms a platinichloride, but no aurichloride, by which again it may be distinguished from adenine. It forms a meta-phosphate that is not soluble in excess of the reagent, while the meta-

phosphate of adenine is soluble. With ferrocyanide in solution with hydrochloric acid guanine forms a crystalline salt, in distinction to xanthine and hypoxanthine. The tests of Strecker and of Weidel give negative results, that with diazo-benzene sulphonic acid positive.

Guanine is converted into xanthine by treatment with nitrous acid (compare page 328). Bacteria and organic extracts effect the same deaminization.

When boiled with strong hydrochloric acid, guanine is decomposed according to the equation:

$$C_5H_5ON_5 + 7H_2O = C_2H_5O_2N + 4NH_3 + CH_2O_2 + 2CO_2.$$
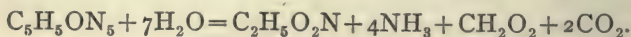
Epiguanine. 7-Methyl-2-amino-6-oxypurine.

Epiguanine is found in human urine following ingestion of tea or coffee. It is extremely insoluble. The crystals melt at 300°. It forms the usual insoluble combinations with silver nitrate and copper. The picrate is very insoluble, also the platinichloride and aurichloride. The Strecker and Weidel reactions are positive.

Hypoxanthine. 6-Oxypurine. $C_5H_4ON_4$.

This base differs from xanthine by an atom of oxygen (see page 321). It separates from its solutions as a white crystalline powder, soluble in 300 parts of cold or 78 of boiling water, and in 900 parts of boiling alcohol. The base is insoluble in ether.

Hypoxanthine forms soluble crystallisable salts with acids. The microscopic appearances of the *nitrate* and *hydrochloride* are characteristic (Fig. 19). The *urate*, which is polymeric with xanthine, is precipitated on adding potassium urate to a solution of hypoxanthine hydrochloride.

The silver oxide compound of hypoxanthine, $Ag_2O,C_5H_4ON_4$, is formed as a gelatinous precipitate on adding ammoniacal silver nitrate to an ammoniacal solution of the base. It is insoluble in ammonia, unless used in great excess, and it dissolves with difficulty in boiling nitric acid of 1.10 sp. gr. On cooling, a compound of the formula $C_5H_4ON_4,AgNO_3$ separates in crystals, which, under the microscope, appear as long prisms or spindles, sometimes isolated but in other cases crossed symmetrically to form stellate groups. The last form is common when the crystallisation occurs slowly. The characters

of the silver nitrate compound allow of the separation of hypoxanthine from other bases of the group (see page 330). Hypoxanthine forms a crystalline *platinichloride*, but not an aurichloride. The nitrate while fairly soluble, is a very crystalline salt, and thus adapted to purification of the base. The *picrate* is soluble in 500 parts of cold water, and forms long sharp needles. The *silver picrate* formed by the mixing of hot solution of the picrate with silver nitrate, is a very insoluble salt.

Hypoxanthine gives negative or only very faint reactions with Strecker's, Weidel's, and Hoppe-Seyler's tests (page 331). After

FIG. 19.

HYPOXANTHINE NITRATE.   HYPOXANTHINE HYDROCHLORIDE.

treatment with hydrochloric acid and zinc it gives a ruby-red colouration on addition of sodium hydroxide in excess. In this reaction it behaves like adenine.

Hypoxanthine is almost always associated with xanthine. It occurs in the flesh and muscles of the heart of the horse and ox, in the pancreas, the spleen, and the liver, especially in cases of yellow atrophy. It has also been found in human and dog's urine. It may be isolated by the method described in the table on page 319, and purified by solution in hot water, with addition of hydroxide of lead, filtration, separation of the lead as sulphide, and concentration of the filtrate to the crystallising point.

The reactions of Strecker and Weidel are negative, that of Kossel is positive. The Kossel test is made by heating a solution in a test tube for $1/2$ hour with zinc chloride and hydrochloric acid. The solution is first filtered, then made alkaline. The presence of hypoxanthine (also adenine, but not guanine and xanthine) leads to the development of a red colour, becoming brown on shaking.

Adenine.   6-Amino-purine. $C_5H_5N_5$.

Adenine was originally obtained by Kossel in treating pancreas for the preparation of hypoxanthine, but is most conveniently prepared from tea. Adenine bears the same relation to hypoxanthine that guanine does to xanthine (see page 321).

When pure, adenine crystallises from its aqueous solution in needles, which dissolve in 1,086 parts of cold water, and are readily soluble in hot water. It is but slighty soluble in hot alcohol, and is insoluble in ether. If crystals of adenine be slowly heated, it will sublime unchanged. But if the heating be done rapidly, it will melt at $365°$, with evolution of gases and slight browning.

Adenine may be obtained in 4-sided pyramids, free from water of crystallisation, by adding excess of ammonia to a concentrated solution of its hydrochloride.

Adenine yields crystallisable salts with acids, and also forms definite compounds with some neutral salts.

Adenine does not give the ordinary colour-reactions characteristic of the xanthine bases, but resembles hypoxanthine in yielding a red colouration on treatment with hydrochloric acid and zinc with subsequent addition of an alkali.

An aqueous solution of adenine (0.5%) gives no precipitate with potassium ferrocyanide or ferricyanide until acetic acid is added, when thin crystalline plates are deposited. With chromic acid adenine forms the compound $(C_5H_5N_5)_2, H_2Cr_2O_7$, crystallising in 6-sided plates. Cupric sulphate produces in adenine solutions an amorphous greyish-blue precipitate, consisting of a mixture of copper-adenine and of adenine-copper sulphate. Ferric chloride gives a red colouration unaltered by heat.

The salt of copper and bisulphite is extremely insoluble. With meta-phosphoric acid it forms a crystalline salt that is soluble in excess of the phosphate. The Strecker and the Weidel tests are

negative, the Kossel and the diazo-benzenesulphonic acid tests are positive. The *oxalate*, the *silver nitrate*, the *picrate*, the *picrolonate* and the *aurichloride* may all serve for isolation and identification. Nitrous acid converts adenine into hypoxanthine, in many ways a useful test.

Adenine and hypoxanthine combine in aqueous solution to form a compound containing $C_5H_5N_5,C_5H_4ON_4+3H_2O$, which crystallises from water in clusters of slender needles which readily effloresce, and rapidly lose water at 100°. The compound forms a homogeneous hydrochloride, which may be separated into its constituents by dissolving it in dilute sulphuric acid and fractionally crystallising.

For the separation of adenine from the allied bases, G. Bruhns (*Ber.*, 1890, **23,** 225) employs the following process: Silver nitrate is added to the nitric acid solution of the bases, when the silver nitrate compounds of adenine and hypoxanthine are precipitated, and xanthine and guanine remain in solution. The precipitate is decomposed by hydrogen sulphide or dilute hydrochloric acid, the resulting solution nearly neutralised by sodium carbonate, and a solution of sodium picrate added. After standing 15 minutes, the precipitate of adenine picrate is filtered off, washed, dried at 100°, and weighed. The original precipitate contains $C_5H_5N_5$, $C_6H_2(NO_2)_3.OH+H_2O$, but becomes anhydrous at 100°, and undergoes no further change below 220°. Adenine picrate is soluble in 3,500 parts of cold water, so that a correction of 2.2 m.grm. must be made for every 100 c.c. of filtrate and wash-water. From the filtrate the hypoxanthine is precipitated by neutralising with ammonia and adding ammoniacal silver nitrate. No correction for solubility need be made if both solutions are free from excess of ammonia.

Adenine is very completely precipitated by cupric sulphate in presence of a reducing agent. By employing sodium thiosulphate as the reducing substance, and operating in a cold solution, separation from hypoxanthine can be effected.

Carnine. $C_7H_8O_3N_4$.

Carnine crystallises in agglomerations of minute irregular crystals. It is very little soluble in cold water, but readily in hot, separating again on cooling. It is insoluble in alcohol or ether. Carnine forms crystallisable salts both with acids and bases. Its *hydrochloride* gives a

golden-yellow precipitate with platinic chloride. The compound with basic lead acetate dissolves in boiling water. The *silver nitrate* compound resembles that of hypoxanthine, and in its reaction with Weidel's test carnine also behaves like hypoxanthine.

Bromine-water is decolourised when added to a boiling solution of carnine. On concentrating the liquid at 100°, brilliant needles of hypoxanthine *hydrobromide* are deposited, and on treatment with caustic soda yield the free base.

The occurrence of carnine in urine is doubtful. Hitherto it has only been found with certainty in extract of meat, of which it constitutes about 1 %. Little is understood of carnine. Indeed, its identity has been denied. It is likely that it is a complex purine, a fore-stage of adenine or guanine. It does not form a picrate, nor are the salts with silver and copper very insoluble; and in many ways it suggests the complex purines, since trimethylpurines have many of the same general reactions.

Methyl-xanthine.   1-Methyl-2-6-dioxy-purine.

This is another of the methyl bases found in the urine after ingestion of tea or coffee. It forms fine white crystals. Crystallised from acetic acid, it forms 6-sided leaves. It is moderately soluble, and the alkali salts are soluble. The *nitrate* and hydrochloride are not very insoluble, but crystallise well. It forms a platinichloride and a aurichloride. The usual insoluble salts with silver and copper occur. The Strecker and the Weidel tests give positive indications.

Isolation of Purine Bases.

The purine bases may be isolated and separated, provided only that enough material is available. With 50 litres of urine a small amount can be separated; with several hundred litres better results are to be attained. For the isolation from tissue, the latter is hydrolysed with hydrochloric acid for several hours, and the filtrate employed for the manipulations.

The material is carried through the precipitation with copper and disulphite as previously described, and carried to the point of crystallisation of the uric acid. The filtrate of this crystallisation contains the purine bases If it is very dark, it may be filtered hot with a little

animal charcoal, but otherwise it is best to avoid this, as a loss is entailed. The solution is then evaporated to a syrup, then taken up in alcohol, again evaporated to a syrup, and the process repeated. This is done to remove the hydrochloric acid. The final concentration is carried to a crumbly powder. This is then washed with cold water until free of hydrochloric acid, then with alcohol and ether. The insoluble fraction contains xanthine, heteroxanthine, guanine and methyl-xanthine; the solution contains adenine, guanine, hypoxanthine, and paraxanthine, with traces of heteroxanthine and methyl-xanthine. The exclusion of exogenous purines from the diet makes the situation much simpler, since then the fractionation is easy.

The bases in the xanthine fraction are dissolved in 3 % hot sodium hydroxide solution, and set aside to cool. Within a day or two, the insoluble sodium salt of heteroxanthine crystallises out, and may be separated and tested as described under that heading. The filtrate is warmed to 60°, and to 6 parts are added a mixture of 2 parts of nitric acid in 2 parts of water. After a day or two, the crystals of xanthine nitrate will crystallise out. The crystals should be collected, dissolved in the least amount of alkali, then diluted to the original volume and treated again as before described. The second crystallisation will usually be pure, and the collected crystals may be tested as previously described. To form the free base, the nitrate should be dissolved in water and evaporated whilst ammoniacal in reaction. The filtrate of the xanthin nitrate is made alkaline with ammonia, and the methyl-xanthine will crystallise out on concentration. If search is made for guanine, this is to be done with the first solution of the xanthine fraction. This is suspended in warm ammonia; on cooling, the guanine goes out of solution, all the others remain dissolved.

The bases in the soluble filtrate, the hypoxanthine fraction, are treated first by addition of ammonia until alkaline. After a day or two the epiguanine (and possibly guanine) will crystallise out. The crystals are to be redissolved and treated with a very slight amount of ammonia, which will throw out epiguanine, but hold the guanine in solution, to be later recovered by strong ammonia. The two crystallisations are to be washed and tested as indicated under the respective headings. The filtrate of the epiguanine is freed from ammonia by heat, and adenine precipitated by careful addition of picric acid in 1 % solution in slight excess. The picrate is filtered off at once, washed, and treated for identification as already indicated. The filtrate is acidified

with sulphuric acid, freed from picric acid by extraction with ether, and the bases then precipitated with copper and bisulphite, as previously described. The precipitate is freed from copper, and the filtrate concentrated, acidified with nitric acid up to 10% and set aside for crystallisation of the hypoxanthine nitrate, which is later collected and tested as previously described. In the filtrate are traces of paraxanthine, with possibly heteroxanthine and methylxanthine. As is obvious, the method is simple (and quantitative as well) in the absence of the methylpurines. And as the usual purposes of the test are to determine the metabolic purines, not the elemination of methylpurines, it is best to exclude all purine from the diet.

If one is estimating the methylpurines, it may be desirable to estimate the unaltered methylpurines that may be eliminated unchanged. Caffeine, theobromine and theophylline are not precipitated by the copper-bisulphite or the silver nitrate treatments. They are therefore contained in the original filtrates. The filtrate, concentrated if necessary, is acidified with sulphuric acid and the bases precipitated with phosphotungstic acid. The precipitate is suspended in cold water, barium hydroxide added to the point of alkalinity, carbon dioxide passed through and the mixture heated. It is filtered hot, and concentrated to a small volume. The residue is then extracted with chloroform, in which the caffeine is soluble, the others not. The theobromine is then precipitated as the double salt of silver nitrate, excess of ammonia being carefully avoided. The caffeine and theobromine may then be secured by crystallisation, in approximately quantitative manner.

PTOMAINES OR PUTREFACTION BASES.

By G. BARGER, M. A., D. Sc.

The ptomaïnes are bases produced in the putrefactive decomposition of animal and vegetable tissues.[1] In nearly all cases they are formed by bacterial action and they do not occur normally in the tissues of animals and of the higher plants; in fungi, however, they are possibly products of normal metabolism, for a number have recently been isolated from ergot and from other fungi.

The term ptomaïne ($\pi\tau\hat{\omega}\mu\alpha$, corpse) is due to Selmi, who in 1870 and 1871 in the course of a medicolegal examination of human corpses, obtained certain bases giving alkaloidal reactions, which bases he was unable to identify with any known vegetable alkaloid. Soon afterwards Gautier observed that substances of a basic nature are produced during the putrefaction of proteins, and in 1881 Gautier and Etard began a more detailed chemical study of these bases by isolating and analysing two of them, which they considered to be derivatives of pyridine. A single analysis of a ptomaïne had, however, already been made by Nencki in 1876; the base $C_8H_{11}N$, which he obtained from the putrefaction of gelatin mixed with ox pancreas, he at first regarded as collidine, but subsequently he considered it to be phenylethylamine and surmised that it was derived from the amino-acid phenylalanine by the elimination of carbon dioxide. This latter important supposition will be referred to below.

In 1883 Brieger began his extensive researches on ptomaïnes; he discovered several new bases, including putrescine and cadaverine, which were soon afterward identified as tetra- and pentamethylene-diamine respectively. Meanwhile, ptomaïnes began to be regarded

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[1] For a more detailed account of putrefaction bases consult L. Brieger, *Ueber Ptomaïne*, Berlin, August Hirschwald, 1885-1886 (3 parts); the article by D. Ackermann, "*Die Isolierung von Fäulnisbasen* in Abderhalden's *Handbuch der bischemischen Arbeitsmethoden*, 1910, vol. II, pp. 1002-1043; the articles on Bases by P. Rona in Abderhalden's *Bioochemisches Handlexicon*, 1911, vol. IV, particularly pp. 801-827; putrefaction bases will also be dealt with more fully by the author in a forthcoming number of the series of *Monographs on Biochemistry*, published by Longmans, Green and Co.

as products of bacterial metabolism and Brieger succeeded in isolating toxic bases from pure cultures of pathogenic organisms (tetanine, tetanotoxine, typhotoxine, etc.). Griffiths also obtained bases from the urine of patients, suffering from various diseases, and Udranszky and Baumann isolated putrescine and cadaverine from the urine in cases of cystinuria.

Of the many ptomaïnes which have at various times been described, but few have characterised with that degree of precision which is required in the case of a new chemical individual. In many cases substances have been described by biologists, who did not possess sufficient chemical training, and the formulæ assigned to these substances are often based on insufficient analytical data. Except in a few cases the constitution of the ptomaïnes remained entirely unknown. Hence it is possible that the same substance has occasionally been described under different names by different authors, or even at various times by the same author, and that many substances which were regarded as pure, were in reality but mixtures. Of late years, however, the great advance which has been made in the chemistry of the proteins has brought with it a corresponding advance in our knowledge of the putrefaction bases. The opinion is gradually gaining ground, that the latter are merely breaking-down products of the proteins and are not formed by any synthetic process, analogous to that which gives rise to the vegetable alkaloids. For this reason the better known putrefaction bases will first be described in relation to the amino-acids from which they are derived; the other ptomaïnes will then be referred to.

Chemistry of Putrefaction.

In the early stages the action of putrefactive bacteria on nitrogenous organic matter, especially on proteins, is quite similar to that which occurs in the alimentary canal of animals and in the higher plants. The proteins are broken down to peptones, and these, in their turn, split up into amino-acids. While in pancreatic digestion, or in germinating seeds, the degradation process stops at the amino-acid stage, bacteria carry it on further, and finally the amino-acids are transformed into ammonia, carbon dioxide, methane, etc. It is by the breaking down of the amino-acids that most of the better known products of putrefaction are formed; this may be illustrated by reference to tyrosine. In putrefaction, tyrosine, or p-hydroxyphenyl-α-aminopropionic acid, $OH.C_6H_4.CH_2.CH(NH_2)COOH$, for the most part loses its nitrogen

and is transformed to p-hydroxyphenylpropionic acid, $OH.C_6H_4.CH_2-CH_2.COOH$, which further yields a series of non-nitrogenous products such as p-hydroxyphenylacetic acid, $OH.C_6H_4.CH_2.COOH$, p-hydroxybenzoic acid, $OH.C_6H_4.COOH$, p-cresol, $OH.C_6H_4.CH_3$, and phenol, $OH.C_6H_5$. It is evident that in this way no ptomaïnes can be formed; we are here more concerned with a second method of degradation, which, in the case of tyrosine is pursued to a much smaller extent than the above-mentioned elimination of the amino-group. In this second process the amino-group is retained, and carbon dioxide is lost instead, so that a base results, p-hydroxyphenylethylamine, $OH.C_6H_4.CH_2CH_2.NH_2$, which for some time at least does not undergo further decomposition and may be isolated from the putrefaction mixture. This elimination of carbon dioxide is conceivable in the case of any amino-acid entering into the constitution of the protein molecule. It was first surmised by Nencki to be the way in which phenylethylamine is formed from phenylalanine, as mentioned above, but the first direct proof that ptomaïnes thus result from amino-acids, was given by Ellinger (*Zeits. physiol. Chem.*, 1900, **29**, 334) who showed that putrescine and cadaverine are formed by decarboxylation from ornithine and lysine respectively. Ackermann has adduced similar evidence in the case of other putrefaction bases and Barger and Walpole have shown that p-hydroxyphenylethylamine in putrefaction is formed from tyrosine.

Although animal tissues consist largely of proteins, small quantities of other nitrogenous substances are also present, and these too may yield putrefaction bases. Thus choline, neurine and especially their decomposition product trimethylamine have been frequently found in decomposing animal tissues; they are derived from the lecithin contained in the latter. Small quantities of creatine, etc., contained in muscle may perhaps also give rise to putrefaction bases.

Physiological Action of Ptomaïnes. Poisonous Food.

Some ptomaïnes, isolated by Brieger and others, were highly toxic, but in general the bases formed by putrefaction are not poisonous; most so-called cases of ptomaïne poisoning are in reality infections with a pathogenic organism. On the other hand, apparently normal food may sometimes contain violent poisons, which, unlike the organisms producing them, are not destroyed in cooking. A notorious example is sausage poisoning ("*Botulismus*") occasionally met with in Germany.

The symptoms resulting from eating poisonous food usually commence with nausea, vomiting, pain in the abdominal region, and diarrhœa, the motions being frequently of an offensive character. The nervous symptoms are commonly faintness, muscular weakness, prostration, and sometimes spasms; followed by fever, headache, and thirst. Convulsions, clonic spasms, dilatation of the pupil, and disturbance of vision, with drowsiness and occasionally coma, are also observed. The *postmortem* appearances are similar to those produced by mineral irritant poisons. The effects of ordinary poisons are commonly manifested within 3 or 4 hours after partaking of the poisoned food, and often in a much shorter time; the symptoms of ptomaïnic poisoning rarely become fully developed in less than 6 to 8 hours, and in some cases are much longer delayed. But in such cases the delay is probably due to the time required for the multiplication of the microorganisms, and points to the great probability of the toxic ptomaïne which they produce being formed after the food was ingested.

Almost the only better known ptomaïne which might account for these intoxications is Faust's sepsine (*q. v.*). Apart from the violent poisoning, a number of characteristic physiological effects have lately been shown to be caused by certain bases which are derived from the amino-acids of proteins by elimination of carbon dioxide. Our chemical knowledge of these bases is greatly superior to that of other ptomaïnes; all these bases have been synthesised and we are equally well informed with regard to their physiological action. For instance, the monamines generally raise the blood pressure on intravenous injection, the action being of a similar type to that of adrenaline; the diamines do not have this action. Of the former, p-hydrophenylethylamine is the most active. Among diamines, β-iminazolylethylamine (4-β-aminoethyglyoxaline), has a powerful action on the uterus Further reference may be made to the detailed description of the individual bases.

Classification of Ptomaïnes.

The classification of ptomaïnes presents some difficulty on account of our ignorance concerning the chemical nature of many of them. Ackermann in Abderhalden's "Handbuch der biochemischen Arbeitsmethoden" has classified them on the Richter system according to their empirical formulæ, but here a plan will be adopted somewhat similar to that used in the second edition of this work. First, all the

better known putrefaction bases will be described in relation to the amino-acids from which they are derived, and then some of the other ptomaïnes will be dealt with.

I. Putrefaction Bases, Certainly or Probably Derived from Amino-acids.

A. Derivatives of Monamino-acids.

Methylamine, $CH_3.NH_2$, has frequently been found, *e.g.*, in putrid fish by Bocklisch (*Ber.*, 1885, **18**, 1922). It is separated by steam distillation; its formation from glycine by loss of carbon dioxide is less probable than its formation from trimethylamine.

Ethylamine, $C_2H_5.NH_2$, obtained from putrid yeast and wheat flour, is perhaps formed in the same way from alanine.

Isobutylamine, $(CH_3)_2CH.CH_2.NH_2$, obtained by Neuberg and Karczag (*Biochem. Zeit.*, 1909, **18**, 434) from the putrefaction of *d-l-α*-amino*iso*valeric acid (racemic valine). Isolated as platini-chloride after steam distillation and extraction of the distillate with ether.

Isoamylamine, $(CH_3)_2.CH.CH_2.CH_2.NH_2$, is doubtless derived from leucine by loss of carbon dioxide. It has been found in cod-liver oil (obtained by the old putrefaction process), in putrid yeast, in putrid horse meat (Barger and Walpole, *J. Physiol.*, 1909, **38,** 343) and in putrid placenta (Rosenheim, *J. Physiol.*, 1909, **38,** 337). The isolation of this base is readily accomplished by utilising its volatility with steam and its solubility in chloroform and ether. By the addition of anhydrous oxalic acid to the dry ethereal solution, the *oxalate*, $C_5H_{13}N.-C_2H_2O_4$, separates out; this salt can be recrystallised from a mixture of acetone and alcohol and after drying in a vacuum, melts at 169°. It is slowly decomposed at 100°. On distillation with lime it yields the free base, which boils at 95° and is readily soluble in water. The *hydrochloride* is extremely soluble in water and therefore somewhat difficult to crystallise, but the *hydrobromide* readily separates out on adding alcoholic hydrogen bromide to an ethereal solution of the base. It forms glistening leaflets, m. p. 225°. 1 c.c. of an $N/10$ solution of an *iso*amylamine salt produces a marked rise of arterial blood pressure in the cat. (See, for a detailed account of the physiological action, Dale and Dixon, *J. Physiol.*, 1909, **39,** 25; compare also Barger and Dale, *J. Physiol.*, 1910, **41,** 25.)

β-**Phenylethylamine,** $C_6H_5.CH_2.CH_2.NH_2$, was supposed by Nencki to be formed from phenylalanine by loss of CO_2, and was obtained by him in the putrefaction of gelatin mixed with ox-pancreas. Various authors have probably described this base as collidine, without proving it to be a pyridine derivative. Phenylethylamine has also been isolated from putrid horse meat (Barger and Walpole, *J. Physiol.*, 1909, **38,** 343). It is a liquid, boiling at 197°, which readily attracts atmospheric carbon dioxide to form a crystalline carbonate. The base is somewhat soluble in water, from which it may be extracted by ether or chloroform. The action of phenylethylamine on the blood-pressure is similar to that of *iso*amylamine, but more powerful. (Barger and Dale, *J. Physiol.*, 1910, **41,** 28).

p-**Hydroxyphenylethylamine,** OH. C_6H_4. CH_2. CH_2. NH_2, is formed from tyrosine by putrefaction (Barger and Walpole, *J. Physiol.*, 1909, **38,** 343), and has been obtained in the autolysis of pancreas (Emerson, *Beitr. Chem. Physiol. Path.*, 1902, **1,** 501) and in the pro-longed peptic digestion of egg albumin (Langstein, *ibid.*, 1902, **1,** 507). In both these cases the digestion mixture was supposed to be sterile. The base was further found by Van Slyke and Hart in Cheddar cheese (*Amer. Chem. J.*, 1903, **30,** 8), by Gautier (*Bull. Soc. Chim.*, 1906, [iii], **35,** 1195) in putrid cod-livers, by Winterstein and Küng (*Zeit. physiol. Chem.*, 1909, **59,** 138) in Emmenthaler cheese, by Barger and Walpole (*J. Physiol.*, 1909, **38,** 343) in putrid horse meat, by Rosenheim (*ibid.*, 1909, **38,** 337) in putrid placenta, and by Barger (*Trans.*, 1909, **95,** 1123) in ergot. This base has been isolated in various ways. It is hardly soluble in chloroform and in ether, but may be shaken out from a sodium carbonate solution by amyl alcohol, but not from a solution in moderately strong sodium hydroxide; thus it is readily separated from non-phenolic bases (Barger and Walpole). The fact that the base is very little soluble in ether, but soluble in amyl alcohol, was also used by Gautier, who crystallised the free base and thus separated it from the homologous "*Tyrosamines*," C_7H_9ON and $C_9H_{13}ON$. It is more convenient, however, to benzoylate by the Schotten-Baumann method; the resulting dibenzoyl-derivative, m. p. 170°, crystallises readily from alcohol (Emerson, Langstein, Rosen-heim, Barger and Walpole). The base has also been isolated as the platinichloride, after preliminary precipitation as phosphotungstate (Winterstein and Küng).

p-Hydroxyphenylethylamine was obtained in small quantities long

ago by heating tyrosine (Schmitt and Nasse, *Annalen*, 1865, **133**, 214). It can also be prepared by the reduction of *p*-hydroxyphenylacetonitrile (Barger, *Trans.*, 1907, **95**, 1127). It has further been synthesised in various other ways (Barger and Walpole, *ibid.*, 1909, **95**, 1720; Rosenmund, *Ber*, 1909, **42**, 4782). *p*-Hydroxyphenylethylamine crystallises from alcohol in hexagonal leaflets, m. p. 161°. It is soluble in 95 parts of water at 15° (Gautier), 10 parts of boiling alcohol, and somewhat less in boiling water. It is slightly soluble in boiling xylene, but almost insoluble in the cold; xylene is a convenient solvent for recrystallisation. The base is, however, most readily purified by distillation, b. p. 161 to 163° under 2 mm. pressure. The base, like tyrosine, gives Millon's reaction and Mörner's reaction. The dibenzoyl-derivative, $C_6H_5.CO.O.C_6H_4.CH_2.CH_2NH.CO.C_6H_5$, melts at 170° and gives Mörner's reaction, but not Millon's reaction. *p*-Hydroxyphenylethylamine is not poisonous, but nevertheless has marked physiological activity (Dale and Dixon, *J. Physiol.*, 1909, **39**, 25). It resembles adrenaline, to which it is also related chemically, and it is the chief cause of the *pressor* action of aqueous ergot extracts. It also acts on the uterus, and was probably the chief cause of the activity of the placental extracts, investigated by Dixon and Taylor (*Brit. Med. Journ.*, 1907, [ii], 1150). Given by the mouth in doses of 30–100 mgm. the base produces in man a slight rise of blood pressure lasting for some hours.

B. Derivatives of Diamino-acids.

Tetramethylenediamine, Putrescine, $NH_2.CH_2.CH_2.CH_2.CH_2.NH_2$, is formed from ornithine, $NH_2.CH_2.CH_2.CH_2.CH(NH_2)COOH$, by putrefaction (Ellinger, *Zeit. physiol. Chem.*, 1900, **29**, 334; Ackermann, *ibid.*, 1909, **60**, 482). Since ornithine is itself a decomposition product of arginine, putrescine is of common occurrence in the putrefaction of proteins. It was first found by Brieger in putrid material of various kinds (see above, page 341). Udranszky and Baumann (*Ber.*, 1888, **21**, 2938) identified it as tetramethylenediamine previously synthesised by Ladenburg, by the reduction of ethylene dicyanide. These authors also obtained putrescine from the urine in cases of cystinuria and isolated it as the dibenzoyl derivative (*Zeit. physiol. Chem.*, 1889, **13**, 562). It is present in ergot (Rieländer, *Sitzler. Gesellsch. Naturw. Marburg*, August 5, 1908) and in cheese (Winter-

stein and Thöny, *Zeit. physiol. Chem.*, 1902, **36,** 28). Putrescine is a liquid, boiling at 156 to 157°, with a piperidine-like odour, also somewhat resembling that of semen. The *dihydrochloride* crystallises in needles, insoluble in absolute alcohol, and only slightly soluble in 96% alcohol (*separation from cadaverine hydrochloride*). The *dipicrate*, m. p. 250°, is sparingly soluble in water; the *dipicrolonate* is sparingly soluble in water and in alcohol, m. p. 263°. The *dibenzoyl derivative* melts at 175°, and is sparingly soluble in alcohol.

δ-*Amino*-n-*valeric acid,* was found by E. and H. Salkowski (*Ber.*, 1883, **16,** 1191) in the putrefaction of fibrin. It probably arises from ornithine (α-δ-diaminovaleric acid) by the elimination of the α-amino group, and is thus derived from the same parent substance as putrescine. δ-amino-n-valeric acid is a much stronger base than the α-amino acids, such as α-amino *iso*valeric acid (valine), with which it is isomeric. The *hydrochloride* forms acicular crystals; the *aurichloride* (m. p. 100°) and *platinichloride* are crystalline.

Pentamethylene diamine, Cadaverine, $NH_2.CH_2.CH_2.CH_2.CH_2.CH_2.NH_2$, a homologue of putrescine, is formed from lysine or α-ε-diamino-n-caproic acid (Ellinger, *Zeit. physiol. Chem.*, 1900, **29,** 334; Ackermann, *ibid.*, 1909, **60,** 482). It was discovered by Brieger in putrid horse meat and other putrid material; it was identified as pentamethylene diamine by Ladenburg, and has frequently been found accompanying putrescine. Thus, together with its lower homologue it was found in ergot (Rieländer, *Sitz. Gesellsch. Naturw. Marburg,* August 5, 1908), in cheese (Winterstein and Thöny, *Zeit. physiol. Chem.*, 1902, **36,** 28) and in the urine in cystinuria (Baumann and Udranszky, *Zeit. physiol. Chem.*, 1889, **13,** 562; Löwy and Neuberg, *ibid.*, 1905, **43,** 338). Werigo (*Pflüger's Archiv*, 1892, **51,** 362) found it in fresh pancreas.

Cadaverine can be transformed to piperidine (Ladenburg, *Ber.*, 1887, **20,** 2216) and conversely it is most conveniently obtained from piperidine, by von Braun's method (*Ber.*, 1904, **37,** 3583). Cadaverine is a syrupy liquid, sp. gr. 0.9174 at 0°, b. p. 175 to 178°, with an odour resembling that of piperidine and of semen. The *dihydrochloride* forms prismatic crystals readily soluble in 96% alcohol (*separation from putrescine hydrochloride*) and yields a *mercury* double salt $C_5H_{11}N_2$, 2HCl, 4HgCl_2, m. p. 214°. The *platinichloride,* $C_5H_{14}N_2.H_2PtCl_6$, is soluble in 70.8 parts of water at 21° and melts at about 215° Gulewitsch, *Zeit. physiol. Chem.*, 1894, **20,** 287). The *aurichloride*, C_5H_{14}-

N_2, $(HAuCl_4)_2$, forms prisms, fairly readily soluble in water, m. p. 186–188°. The *acid oxalate*, $C_5H_{14}N_2.2C_2H_2O_4+H_2O$, leaflets m. p. 143° and the *normal oxalate*, $C_5H_{14}N_2.C_2H_2O_42H_2O$, needles, m. p. 160°, both crystallise from dilute alcohol and are insoluble in absolute alcohol.

The *dipicrate*, $C_5H_{14}N_2.2C_6H_3O_7N_3$, crystallising in needles, m. p. 221°, and the *dipicrolonate*, $C_5H_{14}N_2.2C_{10}H_8O_5N_4$, m. p. 250°, are sparingly soluble in water, the latter also in alcohol. The *dibenzoyl derivative*, $C_5H_{10}(NH.CO.C_6H_5)_2$, melts at 130° and is more soluble in a mixture of alcohol and ether than the corresponding putrescine derivative.

Cadaverine, like putrescine, is not toxic. On intravenous injection it lowers the blood pressure, 10 mgrm., producing a marked effect in the cat (Barger and Dale, *J. Physiol.*, 1910, **41,** 28). A number of bases (saprine, gerontine, neuridine) have been described, which were considered by their discoverers to be isomeric, but not identical with cadaverine. It is very likely, as pointed out by Ackermann in Abderhalden's "*Handbuch der biochemischen Arbeitsmethoden*," 1909, vol. ii, page 1026) that these bases are nevertheless the same as cadaverine.

A synopsis of the properties of these bases, due to Grandis (*Rend. R. Acc. Linc.*, 1890, **6,** 230) is to be found in Guareschi, "Alkaloide," and in the above-mentioned article by Ackermann.

β-**Iminazolylethylamine** (III) (4-β-**aminoethylglyoxaline**) was first obtained by Windaus and Vogt (*Ber.*, 1907, **40,** 3691) from iminazolylpropionic acid (II) which has been synthesised, but is also a degradation product of histidine (I).

| I.
Histidine. | II.
β. Iminazolyl-
propionic acid. | III.
β. Iminazolyl-
ethylamine. |
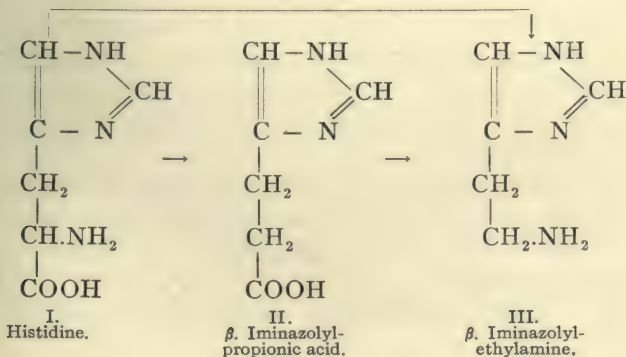
Subsequently Ackermann (*Zeit. physiol. Chem.*, 1910, **65**, 504) obtained the base directly by putrefaction of histidine, from which it is derived by elimination of carbon dioxide. Ackermann's yield was 42% of the theoretical and may even be exceeded, if a pure culture of the right organism is available. Small quantities of the base can also be obtained by heating histidine with mineral acids (Ewins and Pyman, *Trans.*, 1911, **99**, 339), but the amine is best obtained by synthesis, by Pyman's method (*Trans.*, 1911, **99**, 668).

Iminazolylethylamine has been isolated by Barger and Dale (*Trans.*, 1910, **97**, 2592) from ergot, of which it is one of the chief active principles, and they have also found it present in the fresh mucous membrane of the small intestines of the ox (Barger and Dale, *J. Physiol.*, 1911, **41**, 499). The substance was obtained from (fermented) soya beans by Yoshimura (*Biochem. Zeit.*, 1910, **28**, 16) and is probably also present in small quantities in commercial extracts of meat, and of yeast.

β-Iminazolylethylamine has not yet been obtained in the free state. The *dihydrochloride* crystallises from alcohol in prisms and leaflets, m. p. 240°, and is very slightly soluble in cold absolute ethyl alcohol. The *platinichloride*, $C_5H_9N_3.H_2PtCl_6$, forms orange-coloured prisms, readily soluble in water, but hardly at all in alcohol, and decomposing above 200° without melting. The *dipicrate* and the *dipicrolonate* are the most characteristic salts. The former, $C_5H_9N_3.2C_6H_3O_7N_3$, is very sparingly soluble in cold water and when separated slowly forms deep yellow, rhombic leaflets, decomposing at about 235°. The *dipicrolonate*, $C_5H_9N_3.2C_{10}H_8O_5N_4$, only dissolves in about 450 parts of boiling water and crystallises in needles, decomposing at about 261°.

Iminazolylethylamine, like histidine, gives an intense red colouration with a solution of *p*-diazobenzene-sulphonic acid in sodium carbonate (Pauly's reaction). It can, however, be distinguished from histidine, in that it does not react with triketohydrindene (Ruhemann's reagent for α-amino acids).

The physiological properties of iminazolylethylamine have been described by Ackermann and Kutscher (*Zeit. für Biologie*, 1910, **54**, 387) and in greater detail by Dale and Laidlaw (*J. Physiol.*, 1910, **41**, 318 and 1911, **43**, 182).

Agmatine   Guanidylbutylamine, $NH_2.C(:NH)NH.CH_2.CH_2.$

$CH_2.CH_2.NH_2$, has recently been obtained by Kossel from herring spawn (*Zeit. physiol. Chem.*, 1910, **66,** 257) and soon afterward by Engeland and Kutscher (*Zentralbl. f. Physiol.*, 1910, **24,** 479) from ergot. It has not yet been obtained as a putrefaction product, although it might well be so formed (from arginine, by loss of carbon dioxide). It probably breaks down further to putrescine, from which Kossel prepared it synthetically by the action of cyanamide.

Indole ethylamine (*3-β-aminoethylindole*), was quite recently
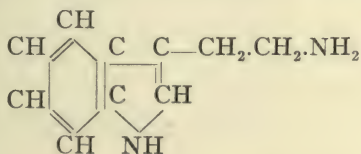
obtained both synthetically and from tryptophane by putrefaction (Ewins and Laidlaw, *Proc. Chem. Soc.*, 1910, **26,** 343, Ewins, *Trans.*, 1911, **99,** 270).

The base is best prepared by heating γ-amino-butyrylacetal and phenylhydrazine with zinc chloride. On removal of the zinc by hydrogen sulphide and concentration of the solution the hydrochloride crystallises. The free base, $C_{10}H_{12}N_2$, melts and decomposes at 145 to 146°, and forms long colourless needles, readily soluble in alcohol and in acetone, but hardly at all in water, ether, benzene or chloroform. The substance gives the blue-violet colouration with glyoxylic and sulphuric acids, described by Hopkins and Cole (*Proc. Roy.Soc.*, 1901, **68,** 21) as characteristic of tryptophane (Adamkiewicz's reaction). The base still gives this reaction at a dilution of 1:300,000. Unlike tryptophane, it does not give a pink colouration with bromine water.

The hydrochloride, $C_{10}H_{12}N_2.HCl$, melts at 246° and dissolves in 12 parts of water at room temperature.

The picrate, $C_{10}H_{12}N_2.C_6H_3O_7N_3$, separates at once on the addition of a saturated aqueous solution of picric acid and forms dark red crystals in fern-like aggregates, resembling ammonium chloride crystals. It is almost insoluble in water, very sparingly soluble in alcohol; m. p. 242 to 243°. The *picrolonate* melts at 231°.

The physiological properties of indolethylamine have been described by Laidlaw, *Biochem. Journ.*, 1911, **6,** 141.

II. Putrefaction Bases not Directly Derived from Amino-acids.

A. Bases of Known Constitution.

Dimethylamine was found by Bocklisch in herring brine, by Brieger in putrid gelatin and in putrid yeast, and by Ehrenberg (*Zeit. physiol. Chem.*, 1887, **11**, 239) in poisonous sausages.

Trimethylamine, $N(CH_3)_3$, is a very common putrefaction product, occurring for instance in herring brine and in aqueous extracts of ergot. It is not formed in the decomposition of proteins, but results from choline, itself a fission product of lecithin. Trimethylamine is best separated by steam distillation and isolated as the aurichloride, $(CH_3)_3N,HAuCl_4$, m. p. 223 to 226°, sparingly soluble in alcohol and in water.

Diethylamine, in cultures of bacteria from poisonous sausages (Ehrenberg, *i.e.*, above).

N-Propylamine, in gelatin cultures of fecal bacteria (Brieger, *Ber.*, 1887, **20,** 797).

Butylamine and **hexylamine** were found (together with *iso*-amylamine) by Gautier and Mourgues in cod-liver oil (*Compt. Rend.*, 1888, **107,** 110).

Neurine, $(CH_3)_3.N(OH).CH=CH_2$, was found by Brieger in putrid meat (*Ber.*, 1883, **16;** 1884, 17) (for description see page 274).

Choline, $(CH_3)_3N(OH)CH_2.CH_2OH$, is readily formed in putrefaction by the decomposition of lecithin. For description see page 276.

Indole and **skatole** occur regularly in the putrefaction of most proteins, and must be derived from tryptophane. They are described on pages 252 and 254.

Methylguanidine, obtained by Brieger from putrid meat, but also present in extract of fresh muscle, is described on page 306.

B. Bases of Unknown Constitution.

In the case of the large number of other putrefaction bases, which have been described, the constitution is unknown and in many cases the empirical formula is doubtful. Only the better known ones will be mentioned.

Neuridine, Saprine, Gerontine.—All these bases have the formula $C_5H_{14}N_2$. They have been regarded as isomeric with cadaverine, to which they show considerable resemblance, but as Ackermann has

pointed out ("Biochemische Arbeitsmethoden," 2, page 1026) it is not unlikely that all these four bases are identical. Whereas cadaverine has been repeatedly found by various observers, neuridine and saprine have only been observed by Brieger and by Bocklisch. Saprine was only found once (by Brieger) and gerontine once, by Grandis, (*Rend. R. Acc. Lincei*, 1890, **6**, 230), who has tabulated the supposed differences between these bases.

Hydrolutidine, $C_7H_{11}N$, isolated by Gautier and Mourgues from cod-liver oil is a liquid, b. p. 199°. It is chiefly of interest on account of its oxidation with potassium permanganate to methylpyridine-carboxylic acid. Thus the base appears to be dimethyldihydropyridine. Similarly a base of the composition $C_8H_{11}N$, obtained by Oechsner de Coninck (*Compt. Rend.*, 1888, **106**, 858 and 1604; 1889, **108**, 58 and 809) from putrid cuttle fish was oxidised by permanganate to nicotinic acid, which on distillation with lime yielded pyridine. These two are the only ptomaïnes for which experimental evidence of the existence of a pyridine ring has been advanced. The evidence of such a ring in ptomaïnes was frequently assumed by early investigators and was doubtless suggested by its occurrence in vegetable alkaloids. It is very probable, however, ptomaïnes have often erroneously been regarded as pyridine derivatives.

Sepsine, $C_5H_{14}O_2N_2$, was obtained in minute quantity from putrid yeast by Faust (*Arch. exp. Path. Pharm.*, 1904, **51**, 248). The base is chiefly characterised by its physiological effects; on intravenous injection it produces in dogs vomiting, diarrhœa, coma and death within 12 hours, without convulsions. The isolation depends on the precipitation of the base by mercuric chloride in the presence of sodium carbonate, and the formation of a crystalline precipitate of the sulphate, when alcoholic sulphuric acid is added to an alcoholic solution of the base. The sulphate, $C_5H_{14}O_2N_2.H_2SO_4$, which was analysed, forms matted needles, which on repeated evaporation of the solution are stated to be transformed into cadaverine sulphate by loss of oxygen. The base is also produced in cultures of *Bacterium sepsinogenes* on agar and in broth.

The formula for sepsine does not appear to be established beyond all doubt.

Viridinine, $C_8H_{12}O_3N_2$, obtained from putrid pancreas by Ackermann (*Zeit. physiol. Chem.*, 1908, **57**, 28) according to Ackermann and Kutscher's method. The *platinichloride*, $(C_8H_{12}O_3N_2)_2H_2PtCl_6$,

forms yellow needles decomposing at 212 to 216°. The *aurichloride*, $C_8H_{12}O_8N_2,HAuCl_4$, crystallises in brownish-black needles, m. p. 176°. The *hydrochloride* forms green needles. Viridine is a mon-acid base.

Marcitine, $C_8H_{19}N_3$, was also obtained from putrid pancreas, by Ackermann (*Zeit. physiol. Chem.*, 1907, **54**, 204) by the same method. The *aurichloride*, $C_8H_{19}N_3.2HAuCl_4$, melts at 175 to 178°.

Putrine, $C_{11}H_{26}O_3N_2$, was isolated together with marcitine. The *aurichloride*, $C_{11}H_{26}O_3N_2,2HAuCl_4$, forms orange-red crystals melting at 109 to 110°.

The following three bases were obtained by Brieger from culture of pathogenic bacteria; they should not be confused with the much more complex toxins, which are not thermostable and are neutralized by anti-toxins.

Tetanine, $C_{13}H_{30}O_4N_2$ was obtained by Brieger by inoculating pure beef-broth with the tetanus bacillus, and found also in human corpses. It is a strongly alkaline yellow syrup, permanent in alkaline solutions but readily decomposing in presence of acids. The hydrochloride is very deliquescent. The *platinichloride*, $C_{13}H_{30}O_4N_2,H_2$-$PtCl_6$, is soluble in water and in alcohol, but is precipitated from its solution in the latter solvent by adding ether. It crystallises in plates which decompose at about 197°. Tetanine is very poisonous, producing tetanic convulsions and death.

Tetanotoxine, $C_5H_{11}N$, is a ptomaïne extracted by Brieger from cultures of the tetanus bacillus, together with tetanine, $C_{13}H_{30}O_4N_2$, and spasmotoxine, a ptomaïne of unknown composition. Tetanotoxine is described as a colourless liquid of disagreeable odour, which can be distilled either alone or with steam. The *hydrochloride* melts at about 205°, and dissolves in water and in alcohol. The *platinichloride* is difficultly soluble and decomposes at 240°, while the *aurichloride* melts at 130° and is readily soluble. Injected subcutaneously, it produces tremor and paralysis, followed by violent convulsions. Isomeric with piperidine.

Typhotoxine, $C_7H_{17}O_2N$, produced by the typhus bacillus, has been described by Brieger. It is a strong base of alkaline reaction, and possesses poisonous properties. The *aurichloride* forms prisms melting at 176°. The hydrochloride and phospho-tungstate are crystallisable. An isomeric *base* was found by Brieger in horse-flesh which had been putrefying four months. It was poisonous, formed no picrate, but

gave a *platinichloride*. The *aurichloride* formed needles or plates melting at 176° and sparingly soluble in water.

Tyrotoxicon is the name given by Vaughan (*Zeit. physiol. Chem.*, 1886, **10**, 146) to a very poisonous crystalline substance extracted by ether from cheese, stale milk and ice-creams. Unfortunately nothing is known about the chemical composition or properties of this substance; it is not even certain whether it is a base.

For the detection of tyrotoxicon in *milk* and *cheese*, V. C. Vaughan recommends the following process (*Analyst*, **8**, 14): The filtrate from the curdled milk, or the filtered cold-water extract of cheese, is neutralised with sodium carbonate, transferred to a separator, and shaken with its own volume of pure ether. The mixture is allowed to stand for 24 hours, or until separation is effected, when the ethereal layer is allowed to evaporate spontaneously in an open dish. The residue is dissolved in water, the liquid again shaken with ether, and the ethereal layer separated and allowed to evaporate as before. Repeated extractions with ether should be avoided, as the purer the tyrotoxicon becomes the less readily is it dissolved.

Arsenical Ptomaïnes.—From the putrefying corpses of animals poisoned with arsenic Selmi extracted ptomaïnes which contained arsenic in a form not readily recognisable, and which gave precipitates with most of the alkaloidal reagents. T. Husemann (*Archiv der Pharm.*, 1881, **219**, 415; *Chem. News*, **45**, 238) has suggested that the *Aqua Tofana* and *Acquetta di Perugia*, which played so important a part in Italian history some centuries ago, owed their intense toxic action to the presence of certain ptomaïnes containing arsenic. According to tradition, the *Acquetta di Perugia* was prepared by rubbing white arsenic into the flesh of a recently-killed hog, and collecting the liquid which dropped therefrom in the course of putrefaction. Highly toxic arsines would not improbably be formed, in addition to which ammonium arsenite and other soluble compounds of arsenic would result, and hence a liquid far more poisonous than a simple aqueous solution of arsenious acid would be obtained.

Selmi has also shown that a volatile arsine is formed by the contact of arsenious oxide and albuminous matters, which possesses a strong toxic action differing somewhat from that of arsenious acid. Husemann has suggested that a similar compound may be formed from the size or paste used for fixing arsenical paper to the wall of a room, and may account for the poisonous effects experienced therefrom.

ANIMAL ACIDS.

By J. A. MANDEL, D. Sc.

The acids occurring in the animal kingdom are in many instances
(*e.g.*, oxalic, palmitic, benzoic) found also in plants, and a large pro-
portion of them have been prepared by artificial means. Hence no
sharp distinction can be drawn between substances of acid function
found in animals and the organic acids from other sources, just as no
sharp distinction can be drawn between animal bases and vegetable
alkaloids. A large number of the acids occurring in the animal
kingdom have been already considered.[1] The acids belonging to the
cyanogen group will be described in the sequel. There remain a limited
number of substances of acid function, which, in their history and
interest, are very closely associated with animal chemistry, and can be
conveniently considered in this work under the heads of "Acids of
Urine," "Acids of Bile," and "Lactic Acids."

Acids of Urine.

Of the acids existing in urine, whether in the free state or as salts,
by far the most important is uric acid (page 391). Hippuric acid
(page 391) exists in the urine of the herbivora, and ornithuric acid
(page 391) in the urinary excrement of certain birds. Glycuronic acid
is a urinary acid of considerable pathological interest. Urine also
contains various ethereal sulphates, but the more important of these
have already been described.[1] Pyrocatechol and other phenolic
substances, sometimes occurring in urine, were described in Volume
III; while the simpler organic acids were considered in Volume I.[1]

Uric acid, 2-, 6-, 8-trioxypurine, $C_5H_4O_3N_4$.

Lactam formula:

$$
\begin{array}{c}
HN-CO \\
|\quad\ | \\
OC\quad C-NH \\
|\quad\ || \quad\ \rangle CO \\
HN-C-NH
\end{array}
\quad \text{or} \quad
$$

Lactim formula:

$$
\begin{array}{c}
N=C.OH \\
|\quad\ | \\
HO.C\quad C-NH \\
||\quad\ || \quad\ \rangle C.OH \\
N-C-N
\end{array}
$$

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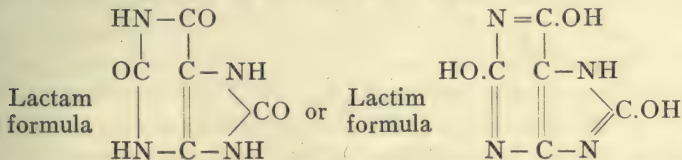
[1] Formic, acetic, butyric, valeric, oxalic, and succinic acids were described in Volume I.
Palmitic, stearic, oleic, cerotic, and other of the higher fatty acids were considered in
Volume II. For phenyl-sulphuric, cresyl-sulphuric, benzoic and hippuric acids, see Volume
III. Indoxyl-sulphuric and skatoxyl-sulphuric acids are described on page 255 *et seq.*, and
oxaluric acid on page 362 of this volume.

Uric acid was discovered by Scheele in 1776, who obtained it from urinary calculi, hence it was called lithic acid until Fourcroy changed the name to uric acid. It was found to be a constituent of the "chalk stone" deposits in the joints, by Pearson in 1798. Uric acid is one of the most constant and characteristic products of tissue metabolism in the human organism. In its formation the nucleic acids of the cell nuclei are especially concerned. It occurs in the urine of all animals, in the excrements of birds, reptiles, scaly amphibians, snails and insects, as well as other invertebrates. The white wings of certain butterflies have been shown, by Hopkins, to contain uric acid. Uric acid also occurs in the blood of birds, but in human blood only under certain conditions. Under pathological conditions it occurs in the blood in pneumonia and nephritis, especially in leukæmia and sometimes also in arthritis. The so-called "chalk stones" and other gouty concretions commonly consist of the sparingly soluble hydrogen sodium urate, while the buff coloured sediment which frequently separates from human urine usually consists of the quadri-urates of sodium or ammonium. Hydrogen ammonium urate constitutes the greater part of the urinary excrement of birds (guano) while that of serpents and other terrestrial reptiles contains it in a still purer form. On the other hand, uric acid is nearly absent from the urine of herbivorous animals, being replaced therein by hippuric acid. The synthesis of uric acid has been effected by Horbaczewski by heating glycocoll with ten times its weight of urea to about 230°:

$$NH_2CH_2COOH + 3CH_4ON_2 = C_5H_4O_3N_4 + 2H_2O + 3NH_3$$
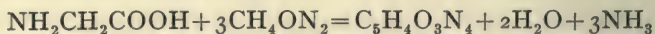
and also by heating trichlorlactic acid amide with an excess of urea

$$CCl_3CHOHCONH_2 + 2CH_4ON_2 = C_5H_4O_3N_4 + 2HCl + NH_4Cl + H_2O.$$
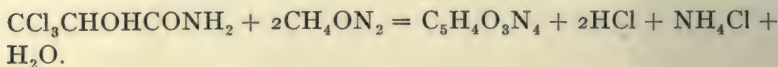
Behrend and Roosen also prepared it from isodialuric acid and urea, while E. Fischer and Tullner prepared it by boiling isouric acid with hydrochloric acid and E. Fischer and Ach obtained uric acid by heating pseudouric acid with oxalic acid to 145°. The above formula shows that uric acid contains the residues of 2 molecules of urea, and explains the fact that the decompositions of uric acid almost invariably yield either a molecule of urea or some derivatives of urea, together with a second substance which can by further treatment be converted into urea. Many of the decomposition-products of uric acid can indeed

be prepared directly from urea. In view of the close relation existing
between urea and uric acid, it is not surprising that the foods which
vn the mammal cause an increased secretion of urea, in birds are con-
erted into uric acid.

Preparation.—Uric acid is best obtained by boiling serpents'
excrement with dilute alkali hydroxide and treating the filtered liquid
with excess of hydrochloric acid. On cooling, uric acid is deposited in
a nearly pure state.[1] Guano may be boiled with a solution of 1 part
of borax in 120 of water, and the filtered liquid precipitated with hydro-
chloric acid. Or it may be first treated with dilute hydrochloric acid
to remove the phosphates, the residue boiled with dilute alkali hydrox-
ide, and the filtered liquid treated with excess of hydrochloric acid.
Another convenient source of uric acid is the yellowish deposit of acid
urates formed on urinals. This may be boiled with sodium hydroxide
as long as ammonia is evolved, carbon dioxide passed through the
filtered liquid, and the precipitated acid urate of sodium washed with
cold water, dissolved in alkali hydroxide, and the solution decomposed
with acetic acid. The product may be purified by dissolving it in hot
alkali hydroxide, boiling with a little potassium permanganate or
dichromate, and filtering the liquid into an excess of dilute hydro-
chloric acid. Uric acid may also be purified by solution in concen-
trated sulphuric acid and precipitation by addition of water.

Uric acid can be obtained from human urine, when free from albumin,
by strongly acidifying the urine with hydrochloric acid and allowing
it to stand in the cold for 24 hours. Dilute urines must first be con-
centrated before acidification. The precipitation of uric acid is not
quantitative and may not occur under certain circumstances. The uric
acid crystals are washed with cold water and alcohol to remove the
pigments as well as benzoic and hippuric acids, which occur as con-
tamination. The crystals can be further purified by dissolving them
in a little sodium hydroxide and then precipitating as acid ammonium
urate, by ammonium chloride, and this decomposed, after filtration, by
hydrochloric acid, or the sodium urate solution is boiled with animal
charcoal and then precipitated by hydrochloric acid. The uric acid
can also be almost completely precipitated from the urine by an
alcoholic solution of picric acid (25 c.c. of a 5% alcoholic solution of

[1] For the isolation of uric acid from birds' excrement, the substance should be boiled with
milk of lime as long as ammonia is evolved. The liquid is filtered boiling hot, when a
filtrate is obtained not more highly colored than urine, whereas sodium or potassium hydrox-
ide gives a highly coloured liquid. From the solution the uric acid is precipitated by
hydrochloric acid.

picric acid for 100 c.c. urine). This precipitate also contains *creatinine*. The washed and dried precipitate is boiled with dilute hydrochloric acid and the picric acid removed from the solution by shaking with ether when the uric acid gradually precipitates.

Properties.—Uric acid forms when pure a crystalline, colourless powder, consisting of rhombic prisms or plates without taste or smell and of a sp. gr. ranging from 1.855 to 1.893. When precipitated from liquids containing urinary pigments or from extracts of guano, the crystals are always yellowish-red or brown, which colour is removed with difficulty by animal charcoal. In rapid crystallisation, small, thin, four-sided, apparently colourless, rhombic prisms are formed, which can be seen only by the aid of the microscope, and these sometimes appear as spools because of the rounding of their obtuse angles. The plates are sometimes six-sided, irregularly developed; in other cases they are rectangular with partly straight and partly jagged sides; and in other cases they show still more irregular forms, the so-called dumb-bells, etc. In slow crystallisation, as when the urine deposits a sediment or when treated with acid, large crystals separate which are coloured. Examined with the microscope these crystals always appear yellow or yellowish-brown in colour. The most common type is the whetstone shape, formed by the rounding off of the obtuse angles of the rhombic plate. The whetstones are generally connected, two or more crossing each other. Besides these forms, rosettes of prismatic crystals, irregular crosses, brown-coloured, rough masses of broken-up crystals and prisms occur, as well as other forms. When deposited from urine or other impure solutions, dumb-bell, whetstone, and lozenge-like forms are among the most common and characteristic (page 381, Fig. 21, *b and c*). Garrod has shown that the pigments of urine are especially concerned in modifying the forms assumed by the uric acid, and that the presence of excess of one particular pigment will produce a corresponding definite variation in the form of the crystals. Edmunds has independently found that the forms assumed by uric acid greatly depend on the nature and amount of the coexisting substances. When precipitated from a solution of a pure urate by addition of hydrochloric acid, uric acid generally forms minute transparent rhombic plates (page 381, Fig. 21, *a*). Large crystals are obtainable much more readily from urine or other impure solutions than from pure urates.

Uric acid is not volatile on heating, and dissolves at 18° in 39,480

parts water (His and Paul) and in 15,505 parts water at 37° (Gudzent). According to His and Paul 9.5% of the uric acid is dissociated in the saturated solution at 18°. Because of the reduction in the dissociation on the addition of strong acids, uric acid is soluble with difficulty in the presence of mineral acids. Uric acid is soluble in glycerin, but it is insoluble in alcohol or ether, slightly soluble in ammonia but readily soluble in alkali hydroxides and in aqueous solutions of certain organic bases such as ethylamine, propylamine, diethylenediamine (piperazin), etc. Uric acid is soluble in neutral solutions of the borates, phosphates, carbonates, acetates, and lactates of potassium and sodium, but not in solutions of the corresponding ammonium salts. In strong sulphuric acid, uric acid dissolves on warming, without decomposition, but when strongly heated with concentrated sulphuric acid, uric acid is broken up and the nitrogen is entirely converted into ammonia. By phosphotungstic acid, in the presence of hydrochloric acid, the uric acid is completely precipitated as a chocolate brown precipitate and it is slowly precipitated by basic lead acetate and also completely precipitated by picric acid. The small amount of uric acid dissolved by ammoniacal solutions is not precipitated by ammoniacal silver nitrate but a gelatinous, flocculent precipitate is obtained in the presence of salts of the alkalies or alkaline earths (magnesia). This precipitate is a double compound of uric acid with silver and these metals (Salkowski).

On being strongly heated uric acid decomposes with the formation of urea, hydrocyanic acid, cyanuric acid and ammonia, and when fused with potassium hydroxide it yields potassium cyanide, potassium cyanate, potassium carbonate and oxalate. When heated with concentrated hydrochloric acid or hydriodic acid in a sealed tube to 170° it splits into glycocoll, carbon dioxide, and ammonia.

By the action of oxidising agents on uric acid a number of compounds of great theoretical interest are obtainable which are mono- or diureides. These form two distinct series. The compounds of the first class, represented by alloxan, $C_4H_2O_4N_2$, are produced by acid-oxidising agents, such as nitric acid. Those of the second class, of which allantoïn, $C_4H_6O_3N_4$, is the type, result from the oxidation of uric acid in alkaline or neutral solution.

By treatment with strong nitric acid in the cold, uric acid yields alloxan and urea: $C_5H_4O_3N_4 + H_2O + O = C_4H_2O_4N_2 + CO(NH_2)_2$. Alloxan forms fine colourless crystals, very soluble in water and alcohol.

The solution has an acid reaction, disagreeable astringent taste, and stains the skin red or purple after a time. Alloxan is decomposed by alkalies, and by oxidising and reducing agents. With ferrous sulphate, it gives a deep blue solution, precipitated on addition of an alkali. By further oxidation, alloxan is converted into parabanic acid, with evolution of carbon dioxide:

$$OC{<}{\begin{array}{c}NH.CO\\\\NH.CO\end{array}}{>}CO + O = OC{<}{\begin{array}{c}NH.CO\\\ \mid\\NH.CO\end{array}} + CO_2$$

Alloxan (mesoxyl-urea).　　　　　Parabanic acid (oxalyl-urea).
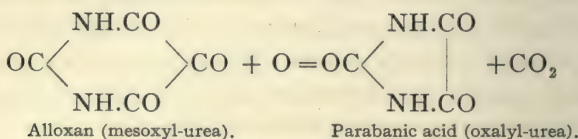
Parabanic acid is also produced by the direct treatment of uric with moderately strong and hot nitric acid. It forms colourless crystals readily soluble in water to a strongly acid liquid. When heated with alkalies parabanic acid assimilates the elements of water and yields oxaluric acid.[1]

Ammonium oxalurate is stated to exist in small quantity in human urine, from which it can be extracted by rendering a large volume (50 litres) of the liquid, faintly alkaline to litmus, filtering from the resultant precipitate, and passing the clear liquid through a moderate quantity of animal charcoal. The charcoal is then washed with cold water till free from chlorides, dried at a gentle heat, and boiled with alcohol. The alcoholic liquid is filtered, evaporated on the water-bath, the residue exhausted with tepid water, and the brownish liquid evaporated to a syrup. On standing in the cold, ammonium oxalurate gradually separates in crystals, which should be washed with absolute alcohol, and recrystallised from boiling water.

If a drop of a solution of pure ammonium oxalurate be allowed to evaporate, the salt appears under the microscope in the form of long-pointed prisms, which reunite to form double hoops or rosettes. If the salt be impure, the hoops remain small and form globules armed with fine needles.

If a solution of ammonium oxalurate be treated with nitric acid, oxaluric acid crystallises out after a time dependent on the concentration. The crystals gradually disappear, and the liquid then contains urea nitrate, the characteristic crystalline forms of which can be ob-

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[1] *Oxaluric acid*, $NH_2.CO.NH.CO.COOH$, forms a white crystalline powder of acid taste and reaction. It is but sparingly soluble in cold water. When boiled for some time with water or dilute alkali it splits into urea and oxalic acid:

$$NH_2.CO.NH.CO.CO.OH + H.OH = NH_2.CO.NH_2 + OH.OC.CO.OH.$$

served by evaporating a drop and examining the residue under the microscope.

A moderately concentrated solution of ammonium oxalurate gives no precipitate with calcium chloride and ammonia, but on heating the liquid it becomes turbid even before the boiling-point is reached, and ultimately an abundant precipitate of calcium oxalate is formed.

Ammonium oxalurate gives no immediate precipitate with silver nitrate, but after a time silver oxalurate separates in fine needles, which do not blacken in the light, and dissolve in ammonia to a solution which is not reduced by boiling.

Hot dilute nitric acid converts uric acid into alloxantin, $C_8H_4O_7N_4$, a substance which is also produced by the action of reducing agents on alloxan. It forms colourless crystals, soluble with difficulty in cold water, but more readily at 100°. The solution reddens litmus, gives with barium hydroxide water a violet precipitate, which turns white and disappears on heating, and reduces silver nitrate. Moistened with ammonia, or exposed to ammoniacal vapors, alloxantin yields a magnificent purple colour, due to the formation of ammonium purpurate or murexide, $(NH_4)C_8H_4O_6N_5$. The formation of this substance furnishes a delicate and characteristic test for uric acid (see page 366).

Chlorine and bromine convert uric acid at ordinary temperatures into urea and alloxan. On heating, parabanic and oxalic acids are also produced. Hypobromites and hypochlorites cause the evolution of a portion of the nitrogen of uric acid in a gaseous state, but the reaction does not appear sufficiently definite to serve as a means of estimating uric acid.

When oxidised in neutral or alkaline solution, by potassium permanganate or ferricyanide, lead peroxide, mercuric oxide or ozone, uric acid yields carbon dioxide and *allantoïn*, $C_4H_6O_5N_4$.[1] By avoiding all rise of temperature, filtering, neutralising with acetic acid, and allowing the liquid to stand for 24 hours, crystals of allantoin are obtained in nearly theoretical proportion. Copper oxide-ammonia oxidises uric acid in the presence of potassium hydroxide to urea and oxalic acid.

Allantoïn is the diureide of glyoxylic acid. It is the characteristic constituent of the allantoïc fluid, especially that of the calf, is found in

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[1] Allantoïn, $OC\begin{cases} NH.CH.HN.CO.NH_2 \\ \quad\quad\quad | \\ NH.CO \end{cases}$

fœtal urine and amniotic fluid, and occurs in the urine of many animals shortly after birth, disappearing at a later period. It appears in the urine after internal administration of uric acid, and has been found in the young leaves of the plane-tree. Allantoïn forms shining colourless prisms of characteristic microscopic appearance (Fig. 20). It is soluble in 160 parts of cold water, more readily in hot water or hot alcohol, but is insoluble in cold alcohol or in ether. Allantoïn has a neutral reaction, combines with metallic oxides, and is soluble in solutions of alkaline carbonates. It reduces Fehling's solution on prolonged boiling and is precipitated by the nitrates of silver and mercury,

FIG. 20.—Allantoïn.

in the presence of small quantities of ammonia, which reactions may be employed for its isolation. With furfural and hydrochloric acid, it behaves like urea but the colouration is less intense and less readily obtained. Allantoïn is best characterised by its crystalline form.

In alkaline solution uric acid takes up water and oxygen and is converted into uroxanic acid, $C_5H_8O_6N_4$, and then into oxanic acid, $C_4H_5O_4N_3$.

As shown by F. and L. Sestini and by Gerard, uric acid can be made to undergo bacterial fermentation with the formation of urea. According to Ulpiani and Cingolani uric acid is quantitatively split into urea and carbon dioxide according to the equation $C_5H_4O_3N_4 + 2H_2O + 3O = 3CO_2 + 2CO(NH_2)_2$. A decomposition of uric acid can also be brought about by the ferments (uricolytic) occurring in many organs such as the kidneys, liver, muscle of the ox and liver of the dog.

The two formulæ given on page 357 are those suggested by Emil Fischer for uric acid, the lactam formula being the ordinary formula, while the lactim formula has the same justification. Fischer believes in the possibility of the existence of the two iso-dynamic forms, basing his belief on the observation that the amorphous acid set free in the cold from its salts has different properties from the crystalline form set free on boiling or on standing for a long time. This supposition of Fischer has recently been proven by the careful physico-chemical investigations of Gudzent (*Centralbt. f. d. ges Physiol. u. Path. d Stoff*,

Lactam-urate (unstable)

	Solubility	1 Litre of solution contains	1 grm. molecule is dissolved in litres.	Specific conductivity	Dissociation	Molecular conductivity $=\infty$	Velocity of migration		Mean value for the anion	Point of saturation is attained in minutes	In 1000 c.c. of a saturated solution	Percentage hydrolysed	The reaction is
							Cations	Anions					
18°													
Prim. potassium urate...	1:477	2.097	99	0.000811	} Calculated					120	$C_{OH} = 6.5 \times 10^{-7}$	0.0064	Faintly alkaline.
Prim. sodium urate.....	1:846	1.182	176	0.000338						45	$= 4.9 \times 10^{-7}$	0.0009	Faintly alkaline.
Prim. ammonium urate.	1:2191	0.456	406	0.000195						15	$= 0.27 \times 10^{-7}$ $= 37.8 \times 10^{-7}$ C uric acid	0.15	Faintly acid.
37°													
Prim. potassium urate...	1:266	3.7585	55	0.002217									Faintly alkaline.
Prim. sodium urate.....	1:469	2.130	98	0.009980						} 15			Faintly alkaline.
Prim. ammonium urate.	1:1225	0.817	226	0.009561									Faintly acid.

Lactim-urate (stable)

	Solubility	1 Litre of solution contains	1 grm. molecule is dissolved in litres.	Specific conductivity	Dissociation	Molecular conductivity $=\infty$	Velocity of migration		Mean value for the anion	Point of saturation is attained in minutes	In 1000 c.c. of a saturated solution	Percentage hydrolysed	The reaction is
							Cations	Anions					
18°													
Prim. potassium urate...	1:716	1.3967	148	0.000540	0.948	84.1	63.8	20.3	} 21	120	$C_{OH} = 5.35 \times 10^{-7}$	0.0079	Faintly alkaline.
Prim. sodium urate.....	1:1270	0.7874	264	0.000225	0.956	63.6	43.3	20.3		45	$= 4.02 \times 10^{-7}$	0.001	Faintly alkaline.
Prim. ammonium urate.	1:3290	0.3039	609	0.000130	0.965					15	$= 0.27 \times 10^{-7}$ $= 25 \times 10^{-7}$ C uric acid	0.15	Faintly acid.
37°													
Prim. potassium urate...	1:402	2.4844	83	0.001462	0.925	131.0	93.2	37.8	} 38.0	120			Faintly alkaline.
Prim. sodium urate.....	1:710	1.4085	148	0.000648	0.933	103.0	65.5	37.5		45			Faintly alkaline.
Prim. ammonium urate.	1:1848	0.5401	342	0.0003709	0.966	131.5	93.5	38.0		15			Faintly acid.

1910, **5,** and *Zeitschr. f. physiol. Chem.,* 1909, **60,** 25, and **63,** 455, which showed that the two forms of uric acid also occur in the living organism and especially play an important rôle in gout. The following table gives the result of Gudzent's observations and emphasises the differences in the two forms of uric acid.

Gudzent's investigations have proven the fact that there are two series of primary salts of uric acid which differ from each other by their solubilities. These investigations have also shown that the first series (α-salts) is unstable and at the moment of their formation in aqueous solution are changed into the second series (β-salts) which are stable, a change which Gudzent explains by an intramolecular rearrangement, corresponding to the two isodynamic forms of uric acid. . (Compare p. 378.)

Detection.

Uric acid is commonly separated in the free state by adding excess of hydrochloric acid to its solution. When separated from urine in this manner, it forms a coloured deposit which adheres to the sides of the glass.[1] The best mode of operating is described in the sequel.

When isolated, uric acid is readily identified by its microscopic appearance, though the forms it assumes are very numerous (see page 382).

A highly characteristic and delicate reaction of uric acid is that known as the "murexide test," which is based on the behaviour of uric acid on oxidation. If uric acid, a urate, or even urine be treated with a few drops of strong nitric acid, and the liquid evaporated to dryness in porcelain at 100°, a yellowish or red residue will be obtained, which owes its colour to the formation of alloxantin, $C_8H_4O_7N_4$. On inverting the capsule over another containing ammonia, or otherwise subjecting the residue to ammoniacal vapours, it acquires a magnificent purple colour, owing to the formation of murexide or ammonium purpurate, $NH_4C_8H_4O_6N_5$. On now adding sodium hydroxide, the purple becomes changed to blue; the colour disappears on warming. An excess of ammonia or sodium hydroxide should be prevented. Somewhat analagous reactions are given by caffeine, theobromine, guanine, and xanthine, but the differences do not allow of their confusion with uric acid.

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[1] A drop of fresh urine, mixed with hydrochloric acid, may be observed under the microscope to deposit uric acid crystals in the course of a few minutes.

The nitric acid prescribed in the above test may be advantageously replaced by bromine-water, or the material to be tested may be evaporated with a few drops of strong hydrochloric acid and a minute crystal of potassium chlorate.

A small quantity of uric acid is heated with dilute nitric acid until effervescence ceases and then the excess of nitric acid is evaporated slowly until a colouration is obtained and this residue treated with 2 or 3 drops of concentrated sulphuric acid to which a few drops of commercial benzene (containing thiophene) have been added. A bluish colouration will be obtained and this changes to brown on the evaporation of the benzene, but becomes blue again on the addition of benzene (Denigès' test).

On dissolving a small portion of uric acid in a little sodium hydroxide, filtering and treating the filtrate with an excess of ammonium chloride, a gelatinous precipitate of ammonium urate occurs immediately or after some time.

If a faintly alkaline solution of uric acid in water be treated with a soluble zinc salt a white precipitate forms which, when collected on a filter, gradually turns blue, owing to oxidation in the presence of sunlight. Potassium persulphate produces an immediate blue colour (Ganassini's reaction).

Uric acid is completely precipitated from its solutions by phosphotungstic acid in presence of hydrochloric acid.

If the uric acid be dissolved in a solution of sodium carbonate, and a drop of the liquid placed on filter paper previously moistened with silver nitrate, a yellow, brown, or black spot will be produced, owing to the fact that silver carbonate is reduced by uric acid even at the ordinary temperature.

On adding a little Fehling's solution to a solution of uric acid in sodium hydroxide, a greyish precipitate is formed, which is said to consist of cuprous urate; but with excess of the reagent, and on application of heat, red cuprous oxide separates, and *allantoïn* is formed. Uric acid does not reduce a hot alkaline solution of picric acid. This fact distinguishes it from creatinine, dextrose, and other normal and occasional constituents of urine which react with Fehling's solution.

For the detection of traces of uric acid in blood or similar liquids, from 150 to 300 c.c. should be boiled, so as to coagulate the proteins, and filtered. The filtrate is evaporated at 100°, and the residue completely exhausted with alcohol. The insoluble portion is then boiled

with water, which will dissolve any urates present.   The solution is cautiously concentrated, and acetic acid added in excess, when uric acid, if present, will gradually separate, and can be recognised by its microscopic characters and its reactions with nitric acid.

If the quantity of liquid available for the test be very small, 5 or 10 c.c. may be treated in a watch glass with 6 to 12 drops of strong acetic acid, and a cotton-thread introduced.   After standing for 24 hours in the cold, microscopic crystals of uric acid may be detected on the thread.

Estimation.

The older methods for estimating uric acid by precipitating it from its solution by the addition of hydrochloric acid and allowing it to stand, yield such inaccurate results that they are not used at the present time.

The method generally employed for the estimation of uric acid is that suggested by Salkowski (*Virchow's Arch.*, **52,** *Pflüger's Arch.*, **5**) and modified by Ludwig (*Wien. med. Jahrb.*, 1884, and *Zeit. anal. Chem.*, **24**).   It consists in precipitating the uric acid from the urine, previously treated with a magnesia mixture and filtered, with silver nitrate and setting the uric acid free from this silver precipitate and weighing or determining in other ways.

The following solutions are necessary:

1. Ammoniacal silver nitrate solution containing 26 grm. silver nitrate in a litre and sufficient ammonia to dissolve the precipitate produced. 2. Magnesia mixture. Dissolve 100 grm. crystalline magnesium chloride and 200 grm. ammonium chloride in some distilled water and add ammonia until it has a strong odour thereof; this is diluted to one litre.   3. A solution of sodium sulphide.   Dissolve 10 grm. sodium hydroxide, which must be free from nitric and nitrous acids, in 1 litre of water.   One-half of this solution is saturated with hydrogen sulphide and then mixed with the other half.   The concentration of these solutions is so adjusted that 10 c.c. of each is sufficient for 100 c.c. of urine.

100 to 200 c.c., according to concentration, of the filtered urine (freed from protein by boiling after the addition of a few drops of acetic acid) is poured into a beaker.   In another vessel mix 10 to 20 c.c. of the silver solution with 10 to 20 c.c. of the magnesia mixture and

add ammonia, and when necessary also some ammonium chloride, until the mixture is clear. This solution is added to the urine while stirring, and the mixture allowed to stand quietly for half an hour. The precipitate which forms is collected on a suction filter, washed with ammoniacal water, and then returned to the same beaker by the aid of a glass rod and jet from a wash bottle, without destroying the filter. Now heat to boiling 10 to 20 c.c. of the alkali-sulphide solution, which has previously been diluted with an equal volume of water, and allow this solution to flow through the above filter into the beaker containing the silver precipitate; wash with boiling water, and warm the contents of the beaker on a water-bath for a time, stirring constantly. After cooling, filter into a porcelain dish, wash the filter with boiling water, acidify the filtrate with hydrochloric acid, evaporate it to about 15 c.c., add a few drops more of hydrochloric acid, and allow it to stand for 24 hours. The uric acid which has been crystallised is collected on a small weighed filter, washed with water, alcohol, ether, carbon disulphide, and again with ether and dried at 100 to 110° and weighed. For each 10 c.c. of aqueous filtrate we must add 0.00048 grm. uric acid to the quantity found directly. Instead of the weighed filter-paper a glass tube filled with glass wool may be substituted (Ludwig). Too intense or too long continued heating with the alkali sulphide must be prevented, otherwise a part of the uric acid may be decomposed.

Salkowski deviates from this procedure by first precipitating the urine with a magnesia mixture (50 c.c. to 200 c.c. urine), filling up to 300 c.c. and filtering. Of the filtrate, 200 c.c. are precipitated by 10 to 15 c.c. of a 3% silver-nitrate solution. The silver precipitate is shaken with 200 to 300 c.c. of water acidified with a few drops of hydrochloric acid, decomposed by hydrogen sulphide, heated to boiling, the silver sulphide precipitate boiled with fresh water, filtered, the filtrate concentrated to a few c.c., treated with 5 to 8 drops of hydrochloric acid, and allowed to stand until the next day.

Another procedure consists in completely removing the washed silver precipitate to a flask of 250 c.c. capacity by means of water and adding 5 to 10 c.c. of a 1% copper sulphate solution, acidifying with a few drops of hydrochloric acid, saturating the liquid with hydrogen sulphide and heating to boiling for a few minutes, filtering and washing with hot water. The filtrate, which must be perfectly clear, is first evaporated in a porcelain dish over a naked flame and then on the water-bath

until it has a volume of a few c.c. and after adding a few drops of hydrochloric acid is allowed to stand for 12 to 24 hours. The deposit of uric acid is collected on a filter and washed as above described, dried and weighed.

Instead of weighing the uric acid the quantity can be estimated from the quantity of nitrogen as determined by the Kjeldahl method or by titrating the uric acid by a standard solution of potassium permanganate (see below). In both cases it is not necessary to collect all the uric acid on the filter as it can be transferred to the digestion flask or to the beaker in which the titration is to be made. In the titration with permanganate it is also necessary to dissolve the uric acid in a little sodium hydroxide (not purified by alcohol) before adding the sulphuric acid.

Folin-Schaffer's method (*Zeit. f. physiol. Chem.*, 1898, **24,** 224 and 1901, **32,** 552) gives the same results as the Salkowski-Ludwig method, although it is simpler. Solutions necessary: 1. A solution of 500 grm. ammonium sulphate, 5 grm. uranium acetate, and 60 c.c. of 10% acetic acid in a litre of water. 2. A 10% ammonium sulphate solution. 3. An $N/20$ potassium permanganate solution obtained by dissolving 1.5815 grm. $KMnO_4$ in water and making up to 1 litre. This solution should be standardized with a $N/20$ oxalic acid solution.

300 c.c. of urine are treated with 75 c.c. of solution 1 and filtered through a folded filter after standing for 5 minutes. By this treatment a biuret-giving substance is removed which interferes in the titration of the uric acid. Place 125 c.c. of the filtrate (corresponding to 100 c.c. of the urine) in a beaker and add 5 c.c. of ammonia and allow to stand 12 to 24 hours. The precipitate of ammonium urate is carefully filtered off and washed by the 10% ammonium sulphate solution. Traces of chlorine do not interfere with the titration, hence the washing is sufficient after 20 to 30 minutes. The precipitate is now quantitatively removed to a beaker by means of a wash-bottle (total 100 c.c.), treated with 15 c.c. concentrated sulphuric acid and titrated at 60 to 63° with $N/20$ potassium permanganate solution until a faint pink colouration is permanent. The temperature must not sink below 50°. Each c.c. of the potassium permanganate solution corresponds to 3.75 mgrm. uric acid. Because of the solubility of the ammonium urate a correction of 3 mgrm. must be added for every 100 c.c. of the urine used.

Hopkins' method is based upon the complete precipitation of the

uric acid as ammonium urate on adding ammonium chloride to complete saturation. 150 c.c. of the filtered urine, whose acidity must not be above 3 c.c. $N/1$ acid for 150 c.c. urine, are warmed in a beaker to 40 to 45° and then 30 grm. ammonium chloride are added and dissolved. After 30 minutes to 1 hour all the uric acid is precipitated as ammonium urate and most will have settled in this time. The cloudy supernatant liquid is decanted into another beaker and the precipitate is first collected on a filter and then the mother-liquor filtered through the same filter. Both beakers are washed with the filtrate until all the urate is transferred to the filter. When this has been accomplished the precipitate is washed with a 10% ammonium sulphate solution until the washings are chlorine free, and then dissolved from the filter by a 1 to 2% sodium hydroxide solution, and the filter then washed with boiling water. The filtrate and wash water are heated on the water-bath until no more ammonia is evolved and then transferred to a Kjeldahl digestor and the nitrogen determined by the Kjeldahl method. 1 c.c. $N/10$ sulphuric acid corresponds to 0.0042 grm. uric acid.

The acid ammonium urate, isolated in the foregoing manner, admits of several alternative treatments, as follows:

1. When it is desired to estimate the uric acid by weight, the precipitate is rinsed off the filter with a jet of hot water, and the liquid heated just to boiling with excess of dilute hydrochloric acid. The liquid is thoroughly cooled and allowed to stand for 2 hours. It is then filtered on to a smooth filter, and the crystals of uric acid washed twice with cold water, then with alcohol till the washings are no longer acid, dried at 100°, and weighed. To the weight of uric acid thus obtained 0.001 grm. should be added for every 15 c.c. ($=1/2$ oz.) of mother liquor, the bulk of which need never exceed 30 c.c.; but no correction need be made for the insignificant trace of uric acid dissolved by the aqueous and alcoholic washings. The uric acid thus isolated is usually only slightly coloured and is practically pure. When derived from highly pigmented urines, the uric acid may retain so much colouring matter as to suggest the presence of an amount of impurity sufficient to vitiate the result. In such case, after washing the precipitate of acid ammonium urate off the filter, rectified spirit equal in bulk to the water present should be added, and, after adding hydrochloric acid, the beaker should be covered and heated for some time on the water-bath.

Instead of weighing the uric acid isolated in the foregoing manner,

it may, if preferred, be dissolved in a little hot solution of sodium carbonate, and the liquid treated by process 3.

2. The precipitate of acid ammonium urate having a perfectly definite composition, may be titrated with standard alkali and an indicator giving no reaction with uric acid. Such an indicator exists in Methyl-Orange. The precipitate from 200 c.c. of urine is treated with a known volume, e.g., 20 c.c., of $N/10$ hydrochloric or sulphuric acid, the liquid boiled for some minutes, cooled, diluted to 200 c.c., a few drops of a 1% aqueous solution of Methyl-Orange added, and $N/20$ sodium hydroxide ($=$ 2.0 grm. of NaOH per litre) dropped in from a burette until the orange colour of the acid liquid becomes yellow, which change indicates the point of neutrality. The difference between the volume of acid employed and that of the alkali required to neutralise it represents the ammonia of the precipitate, uric acid having no action on Methyl-Orange. Each c.c. of $N/20$ solution of sodium hydroxide shows the presence of 0.0084 grm. of uric acid.

3. An alternative plan, when the acid urate of ammonium is not very strongly coloured, is to rinse the precipitate off the filter with hot water, cool the solution, and dilute it with distilled water to 100 c.c. 20 c.c. of pure concentrated sulphuric acid is then added, so as to acidify the liquid and raise its temperature to about 60°, and then a standard solution of potassium permanganate is run in, till the liquid acquires a pink tint surviving agitation and lasting some seconds. Further decolouration may occur on standing, but this should be disregarded. Each c.c. of $1/20$ normal permanganate ($=$ 1.578 grm. of $KMnO_4$ per litre) decolourised represents 0.00375 grm. of uric acid.[1]

F. G. Hopkins strongly recommends this process (*J. Path. and Bacteriology*, 1893, **1**, 451). When it is intended to titrate the acid ammonium urate with standard permanganate in the above manner, it is very desirable to wash the precipitate with a saturated solution of ammonium sulphate, instead of ammonium chloride, since the latter salt somewhat affects the accuracy of the titration.

The same method of titration by permanganate may be applied to the uric acid isolated in process 1 after simply dissolving it in a little hot solution of sodium carbonate.

E. Riegler (*Zeit. anal. Chem.*, 1896, **35**, 35) boils the acid am-

[1] This factor is due to F. G. Hopkins, as the result of experiment. The first results on pure uric acid were not very constant ranging from 96 to 104% of the truth, but taking as the end-reaction the point at which the permanganate ceased to be instantly decolourised, much closer figures were obtained. When the titration was conducted at a boiling temperature, instead of at 60°, the results were higher.

monium urate with Fehling's solution, and estimates the copper in the precipitate obtained.

Urates.

Uric acid is a feeble acid which is usually stated to possess a dibasic function. But it was shown by Bence Jones (*J. Chem. Soc.*, 1862, **15,** 201) and has been confirmed by Sir Wm. Roberts (*Croonian Lectures,* 1892), that a third series of urates exist and have great physiological significance. The salts of the formula $M_2C_5H_2O_3N_4$, commonly called neutral or normal urates, dissolve readily in water, and are exclusively laboratory products, not being met with in the animal system under either healthy or pathological conditions. The acid urates, or "di-urates," of the formula $MH(C_5H_2O_3N_4)$, are very sparingly soluble, and exist in the urine only after it has undergone ammoniacal fermentation. They are known pathologically as components of gouty concretions, in the tissues, but it is questionable if they ever exist physiologically in the blood or tissues. The third class, or "quadri-urates," sometimes called "hemi-urates," have the composition $MH(C_5H_2O_3N_4)H_2(C_5H_2O_3N_4)$. They are more soluble than the di-urates, and are specially the physiological combinations of uric acid. They exist normally in the urine, and probably also in the blood, and constitute the whole of the urinary excretion of birds and serpents. Roberts considers that all the morbid phenomena due to uric acid probably rise from secondary changes in the quadri-urates.

Quadri-urates, $MHUr,H_2Ur$, usually present themselves as amorphous powders, but the "spheres" of birds' and serpents' urine are distinctly crystalline, and display a black cross when examined by polarised light.[1]

These forms are permanent in the air if kept perfectly dry, but readily assume a gelatinous character, and then appear under the microscope as large translucent globules. The quadri-urates are difficult to obtain pure. When produced artificially, they are apt to be di-urates, and when prepared from urine to be contaminated with pigments and traces of extraneous saline matters. Roberts prepares potassium quadri-urate by adding 2 grm. of uric acid to a boiling solution of 9 grm. of potassium acetate in 300 c.c. of water.

[1] Ebstein and Nicolaier (*Ueber die experimentelle Erzeugung von Harnsteinen*, Wiesbaden, 1891).

The liquid is agitated for about a minute, filtered hot, and cooled rapidly in a stream of cold water. The voluminous precipitate which forms is filtered off, washed in succession with rectified spirit and absolute alcohol, and dried at a temperature not exceeding 40°. The results of the analysis of the product obtained agree well with the formula $KH(C_5H_2O_3N_4)H_2(C_5H_2O_3N_4)$. Other quadri-urates can be obtained by similar means, but they are less stable than the potassium salt.

The quadri-urates are insoluble in alcohol, ether, chloroform, glycerol, and volatile oils, and cannot be dissolved without change in any simple menstruum. When treated with hot water, they pass momentarily into solution, but are almost immediately decomposed into bi-urate and free uric acid. The same decomposition is effected by neutral saline solutions, but in this case, and notably with a solution of common salt, the decomposition is greatly retarded. When treated with solutions of alkaline carbonates, or di-sodium hydrogen phosphate, the quadri-urates are converted into di-urates. In healthy urine of feeble acid reaction the quadri-urates dissolve unchanged, but the solution undergoes gradual but complete decomposition, with ultimate separation of the whole of the uric acid in a free state. This change is retarded in normal urine by the salts and colouring matters present (urea has no influence), but occurs with the greater facility the larger the proportion of free acid there is present.

In studying the action of water on quadri-urates, Sir W. Roberts recommends that about 0.4 grm. of the dried deposit should be stirred up with 400 c.c. of distilled water, the mixture heated nearly to the boiling-point until solution is complete, and then left at rest for 48 hours. The supernatant liquid is then syphoned off and the remainder passed through a weighed filter. The crystals of uric acid are washed very sparingly with cold water, then more freely with rectified spirit, dried, and weighed.[1]

A correction of 0.0055 grm. per 100 c.c. of mother-liquor is applied to compensate for the solubility of the uric acid in cold water. The decanted liquid, filtrate, and washings are mixed together, and the mixed liquid heated nearly to boiling, strongly acidified with hydrochloric acid, and allowed to stand 48 hours as before.

By the foregoing method, Roberts obtained the following figures

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[1] It is evident that the modified methods of estimating uric acid, described on page 369, *et seq.*, may with advantage be employed here.

from two specimens of quadri-urate prepared by the potassium acetate process:

	Sample A.	Sample B.
Uric acid separated by water (corrected for solubility),	0.080 grm.	0.164 grm.
Uric acid dissolved as bi-urate,	0.077 grm.	0.159 grm.

These results sufficiently establish the existence of the quadri-urate and the manner of its decomposition by water.

The decomposition of sodium quadri-urate under the influence of water can be conveniently observed by filtering off the buff coloured sediment deposited by healthy urine, washing it thoroughly with cold rectified spirit, and drying it at a blood-heat. When the quadri-urate thus purified is mixed with a considerable volume of water it is speedily disintegrated, a portion passing into solution in combination with the bases, and the remainder falling as an insoluble precipitate of crystalline uric acid. The change is readily observed under the microscope by intimately mixing a particle of the purified deposit on a glass slide with a drop of water and protecting the mixture with a cover-glass. In the course of 10 minutes ovoid leaflets of uric acid make their appearance, and grow and multiply till in the course of half an hour the entire field is thickly studded with crystals; the process continuing, provided that water be added as required, until the amorphous substance is entirely replaced by crystals of uric acid.[1]

The quadri-urates readily assume a gelatinous form. Thus, if a 5% solution of ordinary sodium phosphate be heated to boiling with excess of uric acid, and the liquid filtered hot, the filtrate sets to a jelly on cooling. This jelly, after being pressed between blotting-paper to free it from mother-liquor, exhibits the characteristic behaviour of a quadri-urate, being rapidly decomposed by water with copious formation of crystals of uric acid. On keeping in a moist condition, gelatinous sodium quadri-urate gradually passes into a crystalline condition, and then appears under the microscope in radiating spheres, exactly similar to the spheres so common in serpents' and birds' urine.

If the white mortar-like substance which constitutes the urinary excretion of birds and serpents be examined in its fresh and uncontaminated state, and not after contact with water or bacterial fermen-

[1] Crystals of the sodium di-urate simultaneously formed are never observed, since this salt is liberated in the gelatinous form.

tation, it will be found to behave in an exactly similar manner to artificial quadri-urates. Under the microscope it appears as minute spheres which exhibit a radiated structure and display a black cross with polarised light. On adding a drop of water, the spheres are gradually seen to melt away, with formation of hexagonal tablets of uric acid.

According to W. Roberts, the urinary secretion of serpents and birds consists almost wholly of quadri-urates, and he gives the following analysis of that from the boa, taken from the interior of a massive and very pure specimen:[1]

Uric acid,	82.80%
Potassium,	3.33%
Sodium,	1.06%
Ammonium,	1.92%
Moisture, organic matter, iron, traces of lime, and loss,	10.89%
	100.00
Uric acid as quadri-urates,	80.71%
Uric acid in the free state,	2.09%

Di-urates have the general formula, $MH(C_5H_2O_3N_4)$, or $MHUr$ They result from the action of water on the quadri-urates, and exist in the body under various pathological conditions. The sodium salt, which is the most important and characteristic member of the series, possesses the following properties:

Sodium hydrogen urate, or sodium di-urate, contains $2(NaHC_5H_2O_3N_4)+H_2O$. It generally forms a crystalline powder, which, under the microscope, appears in needles (often crossed), rosettes, stellate and hedgehog-like forms (Fig. 23, page 383). It requires about 1,200 parts of cold or 120 of boiling water for solution. Sodium hydrogen urate is readily obtained by passing carbon dioxide through a solution

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[1] Roberts points out that the boa and other large serpents void their urine at long intervals, varying from a week to six or seven weeks, and that during its long sojourn in the urinary passages the secretion undergoes changes in its composition with liberation of free uric acid. On this account, the uric acid separated by treatment with water is always notably in excess of that which goes into solution as di-urate. This is shown by the following figures obtained by the treatment of freshly gathered specimens of boa's urinary excrement with water:

	A	B	C	D
Uric acid separated by water	0.128	0.110	0.204	0.215
Uric acid dissolved as di-urate	0.117	0.085	0.141	0.142

In addition, contact with water used for cleansing the cages, and bacterial decomposition owing to imperfect desiccation, cause further change of boa's excrement as met with in commerce, so that the article usually met with consists essentially of di-urate of ammonium

of uric acid in sodium hydroxide, or by boiling uric acid with sodium carbonate, phosphate, or acetate, or with borax. The buff or brick-red sediment often thrown down by urine is commonly stated to consist of sodium hydrogen urate, but Roberts has shown that it consists essentially of sodium quadri-urate (p. 373).

The solubility of sodium hydrogen urate in water impregnated with salt and other substances has an important bearing on the cause and cure of gout, and has been investigated by Roberts, from whose results it appears that the solvent action of the various salts depends on the nature of the metal, and has no reference to its form of combination. Salts having an alkaline reaction to litmus, like the carbonates and phosphates, behave exactly similarly to those of neutral reaction, such as the chlorides and sulphates. Salts of potassium exert no appreciable influence on the solubility of the sodium hydrogen urate in water. Salts of sodium decrease in solubility, the influence being greater the larger the proportion of salt present. Salts of ammonium, calcium, and magnesium behave similarly to, but less powerfully than, salts of sodium.

The following figures by Sir Wm. Roberts represent the parts by weight of sodium urate dissolved at 37.8° by 1,000 parts of the solutions of the strengths indicated. The amount of sodium hydrogen urate dissolved by 1,000 parts of distilled water at 37.8° was found to be 1.0.

Percentage of salt in solvent	0.1%	0.2%	0.3%	0.5%	0.7%	1.0%
Sodium bicarbonate	0.50	0.34	0.20	0.13	0.09	0.08
Sodium chloride	0.45	0.30	0.16	0.10	0.08	0.05
Sodium phosphate (crystallised)	0.70	.....	.....	0.32	.....	.....
Sodium sulphate (crystallised)	0.55	.....	.....	0.24	.....	.....
Sodium salicylate	0.65	.....	0.36	0.25	.....	.....
Potassium hydrogen carbonate	0.96	1.00	1.00	0.97	1.02	0.98
Potassium chloride	0.96	.....	1.01	1.10	.....	.....
Potassium phosphate	1.01	.....	.....	1.00	.....	.....
Ammonium chloride	0.85	0.50	0.42	0.35	.....	.....
Calcium chloride	.....	.....	.....	0.27	.....	.....
Calcium sulphate	0.65	0.44	.....	.....	.....	.....
Magnesium chloride	0.85	.....	.....	0.68	.....	.....
Magnesium sulphate (crystallised)	0.90	.....	.....	.....	.....	.....

Crystalline sodium hydrogen urate is ten times as soluble in boiling water as in cold, but a saturated hot solution does not deposit the excess of salt immediately on cooling. The di-urate remains in complete solution for a considerable time, and is not entirely deposited for some days. Roberts has shown that this behaviour is not merely due to supersaturation of the liquid, but is owing to the formation of a

gelatinous modification of the di-urate of greater solubility than the crystalline form. Thus, if a saturated solution of sodium hydrogen urate in boiling water be prepared, and when cold mixed with an equal volume of a 20% solution of common salt, a voluminous gelatinous precipitate will be thrown down. Saturated solutions of solid crystals of other salts (e.g., sodium phosphate or acetate, potassium chloride, phosphate, acetate, etc.) may be substituted for the common salt. The precipitate, if filtered off, allowed to drain, and cautiously washed with cold water, consists of sodium hydrogen urate in a state of approximate purity. It dissolves at 37.8° in blood-serum, or in a liquid containing 0.5 grm. of sodium chloride and 0.2 of sodium carbonate per 100 c.c. (which represents the saline ingredients of serum), sufficiently freely to cause a considerable separation of uric acid after acidifying with acetic acid; whereas crystalline sodium hydrogen urate is taken up by water at 37.8° so slightly that no deposition of uric acid is obtainable on acidifying the liquid (compare p. 366).

The gelatinous form of the sodium di-urate gradually changes into the crystalline variety, and the gradual deposition of the salt from its solution in water, blood-serum, or imitation-serum is evidently due to the same change of condition.

Potassium hydrogen urate, KHUr, is said to be sometimes formed as a urinary deposit in cases of fever. It is amorphous, and is more soluble than the corresponding sodium salt, requiring for solution only 800 parts of cold or from 70 to 80 parts of boiling water.

Lithium hydrogen urate, LiHUr, forms crystalline grains, soluble in 370 parts of cold or 39 of boiling water. Lipowitz states that if equal parts of uric acid and lithium carbonate be treated with 90 parts of water at blood-heat, a clear solution is obtained, while at 100° four times the amount of uric acid can be dissolved without increasing the weight of the lithium carbonate. Seeing that lithium carbonate itself requires about 200 parts of water for solution, its solvent action on uric acid is remarkable, and is of much interest in connection with the extensive application of lithium salts in the treatment of gout. On the other hand, it is stated by L. Siebold (*Year-book Phar.*, 1889, page 413), as the result of direct experiment, that the relative solvent action of solutions of lithium, sodium, and potassium carbonates on a given weight of uric acid, under equal conditions of dilution and at a temperature of 37° (blood-heat), is strictly proportional to the ratio of the molecular weight of these solvents. Hence lithium carbonate has

the advantage that 74 parts are chemically equivalent to 106 of the sodium salt or 138 of potassium carbonate; but there the advantage ceases. Urinary sediments are similarly dissolved by these carbonates with equal facility if molecular proportions are used, and equivalent weights of lithium, sodium, and potassium citrates produce equal alkalinity in the urine of the person taking them. Siebold further states that lithium chloride and sulphate have no solvent action on uric acid and acid urates, and that natural mineral waters containing these salts have none beyond that exercised by basic constituents simultaneously present, and by the water.

Ammonium hydrogen urate, $(NH_4)HUr$, is soluble in about 1,500 parts of cold water, and quite insoluble in saturated solutions of ammonium chloride and sulphate (compare page 371). The urinary excrement of serpents is commonly stated to consist almost wholly of a mixture of ammonium hydrogen urate with free uric acid. This is often true of the altered product, but Sir W. Roberts has shown that, in a fresh undecomposed state, serpents' urine consists substantially of quadri-urates, which undergo decomposition into a mixture of hydrogen urates and free uric acid by contact with water (page 376). Guano, the excrement of various aquatic birds, consists chiefly of oxalate and hydrogen urate of ammonium in admixture with phosphates. Guanine (page 332) is also a constituent of guano, and replaces uric acid in the urine of spiders and other invertebrate animals.

Piperazine urate is described on page 200.

Neutral or normal urates of the light metals do not exist naturally, but they may be obtained by dissolving uric acid in the theoretical amount of alkali. The normal urates of lithium and ammonium are unknown. *Normal potassium urate,* $K_2C_5H_2O_3N_4$, forms small crystals having an alkaline reaction and alkaline taste. It dissolves, with partial decomposition into the acid salt, in about 36 parts of cold water, forming a liquid of soapy taste which froths strongly when shaken.

Normal sodium urate, $Na_2Ur + H_2O$, forms hard nodules which closely resemble the potassium salt, but are less soluble in water.

On passing carbon dioxide through a solution of the normal urate of potassium or sodium the corresponding hydrogen urate is precipitated. The same decomposition occurs by prolonged boiling of the solution, or by its exposure to air. Calcium urate is a frequent constituent of gouty deposits (S. Delépine, *Jour. Chem. Soc.*, 1887, **52,** 469).

The urates of lead, copper, mercury, and silver are quite insoluble in water. Hence solutions of these metals are used for determining uric acid or for separating urates from urine (compare page 368).

The behaviour of the urates with water and saline solutions has an important bearing on the cause and treatment of gout. It is probable that in media containing alkaline carbonates—such as the serum of the blood, and its derivatives, lymph and synovia—uric acid passes into solution in the first instance as quadri-urate, and it may be inferred that it circulates in the blood and is voided in the urine in the same form. In perfect health, the elimination of the quadri-urate proceeds with sufficient speed and completeness to prevent any undue detention or any accumulation of it in the blood. But in gouty subjects, either from defective action of the kidneys or from excessive introduction of uric acid into the circulation, the quadri-urate lingers unduly in the blood and accumulates therein. The detained quadri-urate, circulating in a medium rich in sodium carbonate, gradually takes up an additional atom of base, and is thereby converted into hydrogen urate, which at first exists in the hydrated or gelatinous condition, but with lapse of time and accumulation passes into the insoluble crystalline condition, and then the symptoms of gout manifest themselves.[1]

Urinary Deposits and Calculi.

Urine is sometimes turbid as passed from the urethra, and all urine deposits a fine cloud of mucus on standing. Many specimens of urine, when allowed to cool and stand, deposit urinary salts. These sediments may appear as a purely amorphous or crystalline precipitate or as a mixture of amorphous and crystalline particles.

Urine which is turbid when actually passed from the urethra (and

[1] A. Haig (*Med. Chirg. Trans.*, 71, 125, 183) has shown that administration of acid diminishes the relative amount of uric acid excreted, while that of alkalies increases it. Thus the normal proportion of uric acid to urea is 1:35, but after a few doses of citric acid the relation was 1:41, and after similar doses of potassium citrate, 1:28. In these cases there was not only a relative but also an absolute diminution or increase in the uric acid excreted. Saliclyic acid forms an important exception to the general behaviour of acids, for while it increases urinary activity, it does not in any way diminish the excretion of uric acid. Moreover, acids given while salicylates are present in the circulation have no longer the power of diminishing the excretion of uric acid, nor is excessive excretion of uric acid under salicylates accompanied by any headache. Both uric and salicyluric acids are present in the urine passed under the influence of salicylates, probably owing to the salicylate acting on the uric acid in the blood, but not on that secreted by the kidney itself. Benzoates do not act in the same way as salicylates, probably because the hippuric acid formed from them is less soluble than salicyluric acid. The value of salicylates in uric acid diseases is largely due to their power of preventing acids from causing retention of uric acid. Thus, according to Haig, salicylates prevent gout, the peculiar headache due to uric acid and frequent after breakfast, and also epilepsy, which last affection he believes to be due to uric acid acting on the nerve centres.

consequently at the temperature of the body) may owe its turbidity to the presence of suspended uric acid or urates; to the presence of earthy phosphates or carbonates (especially if the urine be that of a herbivorous animal); or to the presence of organised matters, such as mucus or pus. Deposits which require removal by surgical means from the kidneys, ureters, bladder or urethra, are best considered separately under the head of Urinary Calculi (page 385).

Uric acid and urates are by far the most common and abundant constituents of urinary sediments and calculi.

Urinary Sediments.

Urinary deposits are rarely of a complex character, and hence very simple methods suffice to determine their nature. Examination under the microscope is specially suited for this purpose, since it is simple and readily applied, and is available for a very minute quantity of the sediment. The urine to be examined should be allowed to stand for 12 hours in a conical glass, so that any deposit may collect at the apex, or the urine is placed in a centrifuge and the sediment collected after a few minutes of rotation. A drop of the liquid containing as much sediment as possible should then be withdrawn by a pipette, and placed on a microscope-slide. It should be covered with a thin glass, and examined under an inch objective, which may subsequently be changed for a 4/10 or 1/4 in. objective. Epithelium, mucus

FIG. 21.—Crystals of uric acid a, From decomposition of urates; b, from human urine; c, dumb-bell forms.

globules, and pus cells will be distinguished as organised deposits. Urates and amorphous phosphates appear as opaque particles. Uric acid, which to the naked eye appears as a coloured sandy deposit, is distinguished under the microscope by its peculiar crystalline form and yellow or brown colour. Urine which has been allowed to stand for some time frequently contains a deposit of calcium oxalate, which is seen under the microscope as delicate octahedra. On adding a drop of sodium hydroxide on the slide, a deposit of uric acid will at once dissolve. $N/10$ hydrochloric acid, on the contrary, leaves uric

acid unaffected, or causes a further deposition of minute leaflet crystals under the eye of the observer, while it at once dissolves earthy phosphates or cabonates, whether crystalline or amorphous. The organised deposits are more or less liquefied by alkali, but dilute acid leaves

them unchanged. The addition of a minute amount of staining material, either finely powdered or in solution, at the bottom of the depositing vessel, will stain epithelium and other organised deposits, and thus facilitate their recognition under the microscope.

Uric acid appears under the microscope in a variety of forms. Quadratic prisms, single and in groups, spiculæ, aigrettes, and "dumb-bell" forms are common, as also are somewhat oval crystals attached together so as to form figures of eight, stars, or crosses (Fig. 21). From urine acidified with 5% by volume of hydrochloric acid, square crystals are deposited, having two opposite sides smooth and the alternate sides jagged (Fig. 22). Uric acid crystals dissolve on adding alkali hydroxide, and are reprecipitated in minute but characteristic forms on subsequently adding hydrochloric acid.

Sodium hydrogen urate usually forms amorphous deposits, but sometimes occurs as bundles or tufts of acicular crystals, or in spheroidal masses. (Fig. 23). Potassium and magnesium urates are almost always amorphous. **Ammonium**

FIG. 22.—Crystals of uric acid.

urate occurs only in alkaline urine, and generally in association with magnesium-ammonium phosphate. It forms irregular, club-like crystals or thorn-apple spherules (Fig. 25). Urates are readily distinguished from phosphates by their solubility when warmed in their supernatant urine. When treated with acetic acid, deposits of phosphates dissolve, but urates change

into characteristic forms of uric acid without previously undergoing solution.

Hippuric acid, according to Gorup-Bezanez, is occasionally met with in sediments from the urine of human patients who have taken
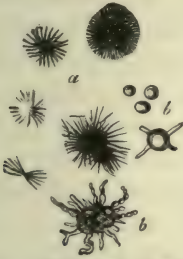
FIG. 23.—Acid urate of sodium. *a*, Needles, usually aggregated; *b, b*, spheroidal masses.
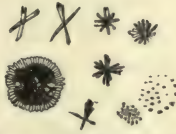
FIG. 24.—Ammonium hydrogen urate.

benzoic acid. It occurs frequently in sediments from the urine of herbivorous animals. Hippuric acid forms characteristic acicular crystals and rhombic prisms (see Fig. 27, page 392). Some of the broader crystals resemble those of ammonium magnesium phosphate, but are insoluble in acetic acid. From uric acid they are distinguished by their solubility in alcohol. If the alcoholic solution be evaporated to dryness, and the residue dissolved in warm water, characteristic crystals of hippuric acid will be obtained on evaporation.

Magnesium ammonium phosphate, often described as "triple phosphate,"[1] is deposited from urine as soon as the liquid becomes alkaline from the decomposition of the urea. The natural deposit may be imitated by adding ammo-
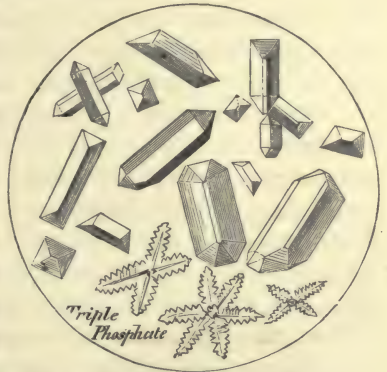
FIG. 25.—Ammonium magnesium phosphate.

nium carbonate to urine, filtering the liquid immediately, and allowing the filtrate to stand for some hours. Magnesium ammonium phosphate forms fine, vitreous, prismatic crystals ("coffin-shaped"), or ragged arborescent or stellate forms (Fig. 25).

[1] The term "triple phosphate" should be abandoned as unscientific and misleading.

Calcium phosphate commonly occurs as an amorphous deposit, which to the naked eye resembles pus or granular organic matter. When precipitated from the urine by heat this deposit has been mistaken for albumin, but is distinguished therefrom by readily dissolving on adding a drop of acetic acid. The same character, and its insolubility in the supernatant urine on warming, distinguish it from deposits of amorphous urates. Under the microscope, amorphous calcium phosphate appears as minute pale granules, arranged in irregular patches. **Magnesium phosphate** has a similar microscopical appearance and character. The crystalline form of calcium phosphate is a comparatively rare deposit. Under the microscope, it appears as crystalline rods, frequently grouped in stars or rosettes, or in club or wedge-like forms, which always show the lines of crystallisation.

Fig. 26.—Crystals of calcium oxalate.

Calcium oxalate usually occurs in minute octahedral crystals in dumb-bell forms (Fig. 26), requiring a 1/4-in. objective for their recognition; it is liable to escape detection unless the phosphates are first dissolved by acetic acid. It occurs in certain morbid conditions, and in the urine of persons after eating rhubarb, sorrel, asparagus or spinach. The deposit may be readily induced by adding a crystal of oxalic acid to normal urine, and allowing the liquid to stand for some hours.

Calcium carbonate is rarely found as a deposit in human urine, but frequently in that of the horse and other herbivora. Under the microscope, the deposit appears as minute spherules or dumb-bells, which show a well defined black cross when viewed by polarised light. It dissolves in warm acetic acid, with effervescence, which may be observed under a low microscopic power, if a number of crystals be treated beneath a large cover-glass.

Calcium sulphate in the form of acicular crystals is said to have been observed as a urinary deposit.

Cystin occurs very rarely as a urinary deposit. It takes the form of hexagonal plates (Fig. 10, page 244), which are soluble in ammonia, and are redeposited in a more perfect form by allowing the resultant solution to evaporate spontaneously.

Leucine (page 227), **tyrosine** (page 231), **xanthine** (page 327), and **cholesterol** (Vol. II.) occasionally occur as urinary deposits.

Organised deposits of various kinds are apt to occur in urine.

Blood-corpuscles, epithelium cells, tube-casts, pus-corpuscles, fat globules, and spermatozoa are all more or less common under certain conditions. Their recognition depends on the employment of the microscopic power of 300 or 400 diameters with the aid of staining agents.

For the more formal chemical examination of urinary deposits, the turbid urine should be warmed to about 50° and filtered at that temperature. Deposits of earthy phosphates, calcium oxalate, uric acid, or organised matters are not dissolved on heating, and hence will be found on the filter, but urates mostly dissolve and are re-deposited from the filtrate in a comparatively pure form on cooling. The uric acid in such deposits may be identified by the murexide test (page 366), while the resultant residue may be ignited and employed for identifying the base. Deposits of urates are often pink or red, owing to the presence of pigment, which may be removed from the deposit by treatment with alcohol.

The portion of the urinary deposit which does not dissolve on warming may be treated with $N/10$ hydrochloric acid, which will dissolve the earthy phosphates without affecting uric acid or the organised deposits. Or the insoluble portion of the deposit may be warmed with dilute acetic acid and the liquid filtered. From the acetic acid solution, calcium may be thrown down by ammonium oxalate. If the filtrate be rendered strongly alkaline with ammonia, a white crystalline precipitate, giving streaks in the track of a glass rod, consists of magnesium-ammonium phosphate. If, after standing for some time, the ammoniacal liquid be filtered and treated with a drop or two of magnesia mixture, and again stirred, a further precipitation of magnesium-ammonium phosphate proves that calcium phosphate was present in the original deposit. The portion of the deposit insoluble in acetic acid may contain uric acid and calcium oxalate. The former is readily detected by the murexide test, by its insolubility in cold hydrochloric acid, and by its microscopic appearance. The calcium oxalate is insoluble in acetic acid, but dissolves in hydrochloric acid, and is reprecipitated on adding excess of ammonium acetate to the resultant solution.

Urinary Calculi.

Concretions, which in the majority of cases consist essentially of uric acid or urates, frequently occur in the bladder, kidneys, and

other parts of the urinary passages. These concretions, or urinary calculi, vary greatly in form, consistency, and composition, and according to their size are known as stones, gravel, or sand. Though sometimes homogeneous, they are more frequently composed of concentric layers, and are always formed on a nucleus, which may generally be distinguished from the adjacent portions and sometimes consists of a foreign substance. Uric acid, ammonium, potassium, sodium, calcium and magnesium urates; iron, calcium, and magnesium phosphates; ammonium, calcium and magnesium carbonates (especially in calculi from herbivora); calcium oxalate; ammonium hippurate; cystin; xanthine; ferric oxide; silica; silicates (in sheep's urine); mucus; blood; colouring and other extractive matters; have all been mentioned as the constituents of calculi. The most common kinds of human urinary calculus are: *a*. Uric acid, with urates of calcium and ammonium; *b*. magnesium ammonium phosphate, with calcium phosphate and carbonate; *c*. uric acid, with phosphates. Calculi consisting essentially of cystin, xanthine, and other compounds, are occasionally, but very rarely, met with.

The following analyses illustrate the percentage composition of some typical kinds of urinary calculus:

Uric acid calculi	A	B
Uric acid..	92.8	84.69
Urates...	3.2	9.03
Ammonium magnesium phosphate...................	....	1.12
Extractive matters..	1.0	2.61
Water...	3.0	

Phosphatic calculus	Oxalate calculus
Sodium urate.................... 9.77	Calcium oxalate................63.5
Calcium phosphate............34.74	Calcium phosphate............. 6.2
Ammonium magnesium phosphate..38.35	Water and organic matters........30.3
Calcium carbonate................ 3.14	
Magnesium carbonate............. 2.55	
Extractive matters................ 6.87	

J. Horbaczewski (*Zeits. Physiol. Chem.*, 1894, **18,** 335) gives the following analyses showing the percentage composition of certain rare urinary calculi:

	Fatty concretion	Cholesterol concretion
Water..	2.5	3.76
Ash..	0.8	0.55
Organic matters insoluble in ether................	11.7	0.15
Organic matters soluble in ether..................	85.0	95.84
Containing:		
Free fatty acids;.................................	51.5	....
Neutral fats.....................................	33.5	....
Cholesterol......................................	traces	95.87

The concentric layers of urinary calculi are frequently distinct in composition as well as in appearance, and a curious alternation of material is at times observed; uric acid, for instance, changing place with urates, phosphates, oxalates, etc. A nucleus of uric acid is generally enclosed with an external coat of phosphates,[1] but the reverse of this appears never to occur. The exterior layers in calculi of various composition are generally phosphatic. The oxalate calculi are usually the hardest, the phosphatic the softest.

Uric acid calculi are very frequently met with. When composed almost wholly of uric acid, a minute portion, heated on platinum foil, chars, burns, and leaves scarcely a trace of ash. Such calculi are usually brownish-red, smooth, or tuberculated, and are composed of concentric laminæ.

Ammonium urate calculus is uncommon. It is clay-coloured, smooth and composed of fine concentric laminæ. This calculus is wholly volatile on ignition.

Cystin calculi are very rare. They are usually small, semi-transparent, smooth, of a greenish or brownish-yellow colour, and insoluble in water, alcohol, or ether. They are soluble in ammonia, and the ammoniacal solution leaves the cystin in hexagonal plates when treated with acetic acid or allowed to evaporate spontaneously.

Xanthine calculus is of very rare occurrence. It is pale brown, of a polished appearance, and soluble in alkaline liquids. On treatment with hydrochloric acid a xanthine calculus yields a solution which on cooling deposits xanthine hydrochloride in hexagonal scales (page 330).

Cholesterol often occurs largely in gall-stones or biliary calculi, but only rarely forms an essential part of urinary calculi. The same remark is true of bile-pigments and bile-acids.

[1] The external layers of phosphates represent a damaged condition of the urinary apparatus consequent upon the growth and presence of the uric acid or other nucleus.

Calcium oxalate often occurs alone, forming a deep red-brown or grey, very hard calculus, tuberculated on the exterior, and called from its appearance "*mulberry calculus*." Smaller and smooth concretions of calcium oxalate often appear as "hemp-seed calculi." Calcium oxalate occurs in large quantities in horse's urine, and often as concretions in pig's urine.

Calcium phosphate occasionally forms concretions of a pale colour, composed of regular laminæ.

Calcium sulphate calculus has been met with in only one recorded case.

Magnesium ammonium phosphate forms white, brittle, and crystalline calculi having an uneven surface. It is seldom laminated, and is not very common.

"*Fusible calculus*" is a mixture of calcium phosphate and ammonium magnesium phosphate. Such calculi are of frequent occurrence, and derive their name from the readiness with which a fragment aggregates and even fuses to a bead when heated on a platinum wire before a blowpipe. The fusibility increases with the proportion of magnesium ammonium phosphate contained in the calculus, calcium phosphate being infusible. Fusible calculi are rarely laminated. They are usually white, soft as chalk, and often are very large.

Analytical Examination of Calculi.

If the calculus be entire, and sufficiently large to allow of the process, it should be sawn in half to ascertain whether it is homogeneous or built up of different concentric layers. If the latter, portions of each layer should be flaked off and examined separately.

A preliminary examination should be made by carefully applying the following tests:

1. Heat a small fragment of the sample on platinum foil, and observe the result. Cholesterol melts and burns freely. Fibrin will give an odour of burnt feathers, and cystin a smell of burning sulphur. If the calculus consists wholly of uric acid, ammonium urate, cystin, xanthine, cholesterol, or other organic matter, it will be entirely volatilised on ignition. Any residue may consist of magnesium oxide, or of potassium or sodium carbonate derived from urates previously existing; calcium carbonate originally existing as such or derived from calcium urate or oxalate; calcium or magnesium phosphate; and traces of silica, oxide

of iron, etc. Any residue left after ignition should be taken up on a loop of platinum wire moistened with hydrochloric acid, and examined in a Bunsen flame for the detection of sodium, potassium, and calcium.

2. Treat a second portion of the calculus with a cold solution of alkali hydroxide. The evolution of ammonia points to the presence of ammonium urate or magnesium ammonium phosphate in the calculus. On adding a few drops of lead acetate to the alkaline liquid and boiling, a black precipitate of lead sulphide will be formed if any cystin were originally present.

3. Treat a third small quantity of the calculus with warm dilute nitric acid. Any effervescence may be due to decomposed urate or to uric acid, but more probably to the presence of calcium carbonate in the calculus. The acid liquid should then be evaporated to dryness on the water-bath. A deep yellow residue points to the presence of xanthine, but its presence should be confirmed by the additional tests described on page 329. Uric acid and urates leave a bright red residue, which on exposure to ammoniacal vapours assumes a magnificent purple tint (page 336).

Careful application of the foregoing tests will generally give adequate information as to the general nature of the calculus, and will suffice to establish the presence or absence of most of the possible constituents. In many cases it is unnecessary to make an exhaustive analysis, but when this is required the systematic process on page 390 may be advantageously employed. It presupposes the calculus to be of the most complex nature, but the results of the preliminary examination will generally allow the procedure to be materially abridged.

Xanthine and *cystine* are occasional, but rare, constituents of urinary calculi. The latter may be detected by boiling a portion of the calculus with alkali hydroxide and lead acetate, and the former by its reaction with nitric acid (page 329). When present xanthine and cystin are precipitated with uric acid when the solution obtained by boiling the calculus with sodium hydroxide is treated with hydrochloric acid. This may be separated from uric acid by treating the precipitate with warm dilute hydrochloric acid. The filtrate, when concentrated and cooled, will deposit the xanthine hydrochloride in crystalline plates. Cystin may be precipitated from the solution as the benzoyl compound (page 246). Or the calculus, preferably previously exhausted in succession with ether, alcohol, and water, may be treated with warm ammonia. On evaporating the ammoniacal solution

Exhaust a weighed quantity of the finely-powdered calculus (placed in a small plaited filter) with ether in a Soxhlet extractor.

Ethereal Extract is evaporated. The residue, dried at 100° and weighed, consists of *cholesterol, fatty matters,* and certain *resinous biliary matters,* the weight of which can be ascertained by weighing the residue left on evaporation. Cholesterol will be deposited in crystalline plates having the characteristic angles (79° 30′ and 100° 30′). To separate cholesterol from fatty matters, etc., saponify with alcoholic potassium hydroxide, boil off the alcohol, add water, and shake with ether, which dissolves the cholesterol only. The alkaline liquid acidified with dilute sulphuric acid gives *fatty and resinous acids,* which can be shaken out with ether and recovered by evaporation.

Residue. Exhaust with hot rectified spirit, preferably without removing it from the Soxhlet extractor.

Alcoholic Extract may contain hippuric acid, bile-pigments, and other colouring matters, the weight of which can be ascertained by weighing the residue left on evaporation.

Residue. Remove from the Soxhlet-tube, boil with a moderate quantity of water, and filter hot. If the filtrate gives a deposit on cooling, the residue should be boiled again with water, but not otherwise.

Aqueous Extract. Evaporate to small bulk, add excess of hydrochloric acid, and allow to stand in for six hours. Filter, and wash deposit with a little cold water.

    Precipitate, washed with alcohol to remove colouring matter, consists of *uric acid* existing in the calculus as *urates.* It may be dried at 100° and weighed, or the uric acid may be estimated, without previous precipitation by hydrochloric acid, by the methods on page 371, *et seq.*

    Filtrate. Divide into two equal portions.
    I. Distil with lime, and titrate distillate with standard acid. The *ammonia* found existed in the calculus as *ammonium urate.*
    II. Evaporate to dryness. The residue consists of NaCl (with perhaps KCl and MgCl₂, etc.), corresponding to the *urates* of the calculus.

Residue. Treat with warm dilute sodium hydroxide, and filter.

    Filtrate. Add excess of acetic acid, keep cold for some hours, and filter.

        Precipitate consists of *uric acid* existing as such in the calculus, and in rare cases of *xanthine* and *cystin* (see page 389).

        Filtrate. Divide into two equal portions. To one portion add potassium ferrocyanide. If a white flocculent precipitate be formed, due to *proteins,* evaporate remaining portion of solution to dryness, heat residue at 100° till free from acetic acid, and add water. The insoluble matter consists of *mucus* and *albuminous matters.*

    Residue. Wash with a little cold water and treat with warm acetic acid. Effervescence indicates *calcium carbonate.* Dilute the liquid and filter.

        Residue. Boil with a solution of carbonate, and filter.

            Residue consists of CaCO₃ from decomposition of calcium oxalate, together with oxide of iron, silica, etc.

            Solution. Acidify with acetic acid, add CaCl₂, digest, and filter. Precipitate, washed, dried at 130° and weighed, represents the *calcium oxalate* of the original calculus. On strong ignition it gives the CaO existing as oxalate.

        Solution. Add ammonium oxalate, digest, and filter.

            Precipitate of CaC₂O₄ represents the Ca existing in the calculus as *carbonate* and *phosphate.*

            Filtrate. Add excess of ammonia and filter.

                Precipitate washed with ammonia water represents the *ammonio-magnesium phosphate* of the calculus.

                Filtrate. Divide into two equal parts.
                I. Add magnesia mixture. Ppte. represents phosphate existing as *calcium phosphate.*
                II. Add sodium phosphate to ppte. remaining *magnesia,* if any.

nearly to dryness the cystin is deposited in crystalline tables, or it may
be precipitated by somewhat concentrating the ammoniacal solution
and adding excess of acetic acid. Xanthine may be isolated and pre-
cipitated in the same manner. Its co-occurrence with cystin has not
been observed, so that no separation of the two substances is
necessary.

Hippuric Acid.—Benzoyl-aminoacetic acid, Benzoylglycine, C_9H_9-
O_3N; *i.e.*, $C_6H_5CO.NH.CH_2COOH$.

Hippuric acid affords a typical example of the so-called "conjugated
bodies," the synthesis of which is readily effected within the living
organism. Thus, if benzoic acid be taken internally, it appears in
the urine as hippuric acid, and hippuric acid may be obtained artificially
by heating benzoic anhydride with aminoacetic acid (glycine), or the
zinc salt of the latter with benzoyl chloride:

$$C_6H_5COCl + CH_2NH_2COOH = C_6H_5CO.NH.CH_2COOH + HCl$$

<div style="margin-left:2em">Benzoyl   Glycocoll.   Hippuric acid.
chloride.</div>
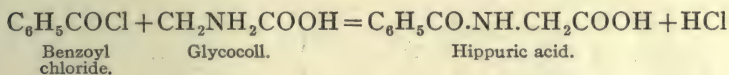
or by the action of chloracetic acid upon benzamide:

$$C_6H_5CO.NH_2 + CH_2ClCOOH = C_6H_5CO.NH.CH_2COOH + HCl$$

<div style="margin-left:2em">Benzamide.   Chloracetic acid.   Hippuric acid.</div>
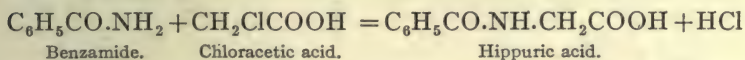
Benzoic aldehyde, toluene, cinnamic acid, quinic acid, and phenyl-
proprionic acid when ingested, are also excreted as hippuric acid.
Substituted benzoic acids appear in the urine as substituted hippuric
acids. (See Salicyluric Acid, page 395.)

The quantity of hippuric acid excreted in normal human urine is
stated to range from 5 to 60 grains (0.3 to 3.8 grm.) in 24 hours, but
an increase results from a vegetable diet. This has been particularly
noticed after eating plums, pears, and cranberries, and the cuticular
parts of many plants act similarly. In the urine of diabetic patients,
hippuric acid is frequently present in much increased proportion, as
also in jaundice and other liver complaints, and it is abundant in
the acid urine of persons suffering from all kinds of fevers.

Hippuric acid replaces uric acid in the urine of herbivorous animals,
which are stated to contain it to the extent of about 2%; its origin being
doubtless in substances of the aromatic series existent in the food.
Hippuric acid is also found in the excrement of the lower animals,
except that of birds, which contains the allied substance *ornithuric
acid*, having the constitution of a dibenzoyl-diaminovaleric acid:

$$C_6H_5CO.NH.CH_2.CH_2$$
$$\Big| \atop CH_2$$
$$C_6H_5CO.NH.CH\diagdown^{CH_2}_{COOH}$$
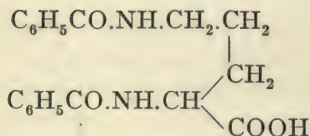
On boiling ornithuric acid with hydrochloric acid, it almost immediately parts with one benzoyl-group and yields benzoyl-ornithine, which on further boiling splits up into benzoic acid and diamino-valeric acid or ornithine, $(NH_2)_2C_4H_7COOH$, a base of strong alkaline reaction and of caustic taste.

When boiled for a time (half an hour) with dilute nitric, hydrochloric, or oxalic acid (or more rapidly if strong hydrochloric acid be used), hippuric acid undergoes hydrolysis, the liquid on cooling de-

FIG. 27.—Hippuric acid (after Frey). a, a, Prisms; b, crystals formed by slow evaporation.

FIG. 28.—Benzoic acid.

positing benzoic acid, while a salt of glycocoll remains in solution $C_9H_9O_3N + H_9O = C_7H_6O_2 + C_2H_5O_2N$. This reaction is employed in practice for the preparation of glycocoll. A similar reaction takes place spontaneously in urine containing hippuric acid under the influence of ferments. Hence only perfectly fresh urine will yield hippuric acid. If the urine be alkaline, as is usually the case with that of herbivorous mammals, the glycocoll first produced is further changed.

Hippuric acid is distinguished from benzoic and salicylic acids by its crystalline form (Figs. 27 and 28); by charring when heated with strong sulphuric acid; by giving off ammonia on ignition with soda-lime; and by not being dissolved on agitating its solution with chlo-

roform or petroleum spirit. When heated, benzoic acid sublimes un-
changed; but hippuric acid gives red oily drops and evolves an odour
of hydrocyanic acid. When precipitated by hydrochloric acid, hip-
puric acid separates immediately in needles, whereas benzoic acid
forms scales. Hippuric acid is less soluble than benzoic acid in ether.
From the neutral solution of a hippurate a neutral solution of ferric
chloride throws down ferric hippurate as a cream-coloured precipitate,
whereas the precipitate yielded by a benzoate with ferric chloride is
reddish-brown.

Preparation.

Fresh horse or cow urine is boiled for a few minutes with an excess
of milk of lime, filtered while hot, concentrated and the cold syrup
precipitated by an excess of hydrochloric acid. The hippuric acid
precipitate is collected and pressed dry on filter-paper, dissolved in
milk of lime by the aid of heat and this concentrated solution pre-
cipitated again by hydrochloric acid. The crystals are further
purified by recrystallisation or by decolourisation with animal charcoal,
if necessary.

Properties and Reactions.

Hippuric acid crystallises in semitransparent, long four sided, milk
white rhombic prisms or columns or in needles by rapid crystallisation.
They dissolve in 600 parts cold water but more easily in hot water.
They are easily soluble in alcohol but with difficulty in ether. The
acid dissolves more easily (about twelve times) in ethyl acetate than
in ethyl ether. Petroleum benzin does not dissolve hippuric acid.

Hippuric acid melts at 187.5° to an oily liquid which crystallises on
cooking. On continuing the heat it decomposes, producing a red
mass and a sublimate of benzoic acid, with the generation first of a
peculiar pleasant odour of hay and then an odour of hydrocyanic acid.
On evaporation to dryness with nitric acid an intense odour of nitro-
benzene is obtained when the residue is heated with sand in a glass
tube (Lücke's reaction). For the detection of hippuric acid, K. Spiro
(*Zeitschr. f. Physiol. Chem.*, 1899, **28**, 174) recommends warming the
hippuric acid with acetic anhydride, anhydrous sodium acetate and
benzaldehyde. After warming for 1/2 hour the lactimide of phenyl

aminocinnamic acid crystallises out and has a melting-point of 165 to
166°.   On heating the lactimide with strong sodium hydroxide until
ammonia is given off and acidifying, phenylpyroracemic acid,
$C_6H_5CH_2CO.COOH$, separates out.   This acid is soluble in ether.

Estimation.

The quantitative estimation of hippuric acid in the urine may be
performed by the following method of Bunge and Schmiedeberg
(*Arch. f. expt. Path. u. Pharm.*, 1877, **6**, 233): At least 300 c.c.
human urine is first made faintly alkaline with sodium hydroxide,
evaporated nearly to dryness, and the residue thoroughly extracted
with strong alcohol.   After the evaporation of the alcohol the residue
is dissolved in water, the solution acidified with sulphuric acid, and
completely extracted by agitating (at least five times) with fresh
portions of ethyl acetate.   The ethyl acetate is then repeatedly washed
with water, which is removed by means of a separating funnel, then
evaporated at a moderate temperature and the dry residue treated
repeatedly with petroleum-ether, which dissolves the benzoic acid,
oxyacids, fats, and phenols, while the hippuric acid remains undissolved.
This residue is now dissolved in a little warm water and evaporated
at 50 to 60° to crystallisation.   The crystals are collected on a small
weighed filter.   The mother-liquor is repeatedly shaken with acetic
ether.   This last is removed and evaporated; the residue is added to
the above crystals of the filter, dried and weighed.

Völcker's Method (*Maly's Jahresb.*, 1887, 215).—200 to 300 c.c.
urine are evaporated in a Hofmeister dish to one-third of its volume
and then to a syrupy consistency after the addition of 4 grm. sodium
phosphate and after mixing with plaster of Paris, the mass is heated
until it becomes a dry powder.   The entire powder, with the broken
dish, is now extracted in a Soxhlet extraction apparatus first for
6 to 8 hours with petroleum ether and then for 6 to 10 hours with
dry ether.   The ethereal residue is dissolved in water, the solution
decolourised with animal charcoal, evaporated at 50 to 60° to 0.5 c.c.
volume and allowed to crystallise.   The crystals are collected on a
weighed filter, dried at 110°, washed with water and a few drops of
ether, dried and weighed.   A correction of 0.0015 grm. hippuric acid
must be made for every c.c. of filtrate.

Methods of estimating hippuric acid have also been suggested by

Jaarsveld and Stokvis (*Arch. of expt. Path. u. Pharm.* 1879, **10,** 268, and 1883, **17,** 189), and by Wiechowski (Hofmeister's Beiträge 1906, **7,** 204).

Salicyluric acid, $C_9H_8(OH)O_3N$, has the constitution of a hydroxyhippuric acid. It occurs in the urine after administration of salicylic acid, which has the constitution of ortho-hydroxybenzoic acid, and may be detected therein by the bluish-violet colouration produced on adding dilute ferric chloride. Salicyluric acid is more soluble than hippuric acid. On boiling with hydrochloric acid, it is split up into salicylic acid and glycocoll.

Glycuronic acid (also *glucuronic acid*), $C_6H_{10}O_7$; *i.e.*, COH-$(CHOH)_4COOH$. Glycuronic acid doubtless has its origin in the dextrose of the body, to which compound it is closely related.[1]

It was first obtained in the conjugated form of campho-glycuronic acid in the urine of dogs to which camphor had been administered, and subsequently as uro-chloralic acid after the administration of chloral. Glycuronic acid is remarkable for its tendency to form ethereal or glucosidal compounds when appropriate substances are introduced into the body. Traces of such compounds occur normally in urine, especially indoxyl- and skatoxyl-glycuronic acids; in addition to the combination with urea, having probably the constitution of uroglycuronic acid, which appears to be the ordinary forms in which glycuronic acid exists in urine.

Baeyer (*Annalen*, 1870, **155,** 257) has shown that euxanthic acid, which exists in combination with magnesia in the "purrée" or "Indian yellow" of commerce,[2] is decomposed on boiling with hydrochloric acid or dilute sulphuric acid, with formation of euxanthone and an

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[1] The relation between glycuronic acid and substances of the sugar-group is shown by the following constitutional formulæ:

Dextrose	$CH_2(OH).(CH.OH)_4.CO.H$
Gluconic acid	$CH_2(OH).(CH.OH)_4.CO.OH$
Saccharic acid	$CO(OH).(CH.OH)_4.CO.OH$
Glycuronic acid	$CO(OH).(CH.OH)_4.CO.H$
Gulonic acid	$CO(OH).(CH.OH)_4.CH_2.OH$
Gulose	$CO(H).(CH.OH)_4.CH_2.OH$

[2] Piuri or Purrée, now used as a pigment under the name of "Indian yellow," is obtained in Bengal from the urine of cows which are fed exclusively on the leaves of the mango tree and water. The urine is heated, and the precipitate separated and dried. Analyses of very pure specimens of purrée by C. Graebe (*Annalen*, **254,** 265) showed: euxthanic acid, 51; silica and alumina, 1.5; magnesia, 4.2; lime, 3.4; and water and volatile substances, 39%. The analyses of Stenhouse and Erdmann show much less lime. Urea, uric acid, and hippuric acid have also been found in purrée. The poorer qualities contain considerable quantities of euxanthone, partly free and partly in combination. For the isolation of the euxanthic acid and euxanthone, and the assay of purrée, the colouring matter should be triturated with dilute hydrochloric acid until the whole has assumed the bright yellow colour of euxanthic acid. The residue is then well washed with cold water to remove the salts, and the euxanthic acid extracted from the residue by ammonium carbonate solution. It is precipitated from the filtrate by hydrochloric acid, and purified by crystallisation from alcohol. The euxanthone, left undissolved by the ammonium

acid which has been shown by Spiegel (*Ber.*, 1882, **15,** 1965), to be identical with glycuronic acid, $C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$. In fact purrée is the best material for the preparation of glycuronic acid, which can be obtained on the small scale by the following process: The artists' water-colour known as "Indian yellow" is ground up with sand, and then treated with dilute hydrochloric acid, which dissolves out calcium and magnesium salts, etc. The residue is washed with water and treated with a solution of ammonium carbonate, which dissolves the euxanthic acid, leaving euxanthone and sand undissolved. From the filtered liquid the euxanthic acid is precipitated by dilute hydrochloric acid, washed with cold water, and then heated with water in a closed soda-water bottle to 125° for 3 or 4 hours. The requisite temperature can be conveniently obtained by immersing the bottle in a bath of molten paraffin. From the cooled product the euxanthone is dissolved by agitation with ether, and the glycuronic anhydride crystallised from the concentrated aqueous liquid.

According to Neuberg (*Ber.*, 1900, **33,** 3315) the euxanthic acid obtained on treating the purrée with hydrochloric acid is suspended in 10 times its weight of water and heated in an autoclave to 135° with one-half its weight of $N/2$ sulphuric acid for 3 hours. After 12 hours' standing the euxanthone is sucked off on a Buchner filter, and the sulphuric acid removed by barium hydroxide solution and the filtrate evaporated in a vacuum. On adding the residue to a large quantity of boiling alcohol the barium glycuronate goes into solution and the reversion products precipitate. The alcoholic filtrate is evaporated to a syrup in a vacuum and crystals will form in 1 to 2 days. These are dried on unglazed porcelain plates and recrystallised from hot ethyl acetate on adding some absolute alcohol.

Glycuronic acid is a syrupy liquid, miscible with water or alcohol. When the aqueous solution is boiled, evaporated, or even allowed to stand at the ordinary temperature, the acid loses the elements of water and yields the anhydride or lactone.

carbonate, is treated with sodium hydroxide, the solution precipitated with an acid and the precipitated euxanthone shaken out with ether or filtered off and dried at 100°.

Euxanthic acid has the constitution:

$$OH.C_6H_3\Big\langle{}^O_{CO}\Big\rangle C_6H_3O.CH(OH).(CH.OH)_4COOH.$$
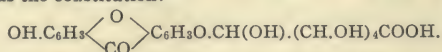
It forms pale yellow needles, m. p. at 156 to 158°. It has a sweet taste and bitter after taste, is but slightly soluble in cold water, very sparingly in ether, but readily in boiling alcohol. Alkalies colour the solution deep yellow. Euxthanic acid does not reduce Fehling's solution, nor form a compound with phenyl-hydrazine.

Euxanthone is a neutral substance, crystallising in pale yellow needles, soluble in alkalies, but not in dilute acids. It forms no compound with phenyl-hydrazine. (See Vol. V.)

Glycuronic anhydride, $C_6H_8O_6$, forms monoclinic tables or needles, having a sweet taste, and m. p. 160° when heat is gradually applied, or at 170 to 180° when heated rapidly. The anhydride is insoluble in alcohol, but dissolves readily in water to form a dextrorotatory solution $[\alpha]_D = 19.25°$. The solution prevents the precipitation of cupric solutions by alkalies, and powerfully reduces Fehling's solution, the copper reducing power being 98.8, compared with glucose as 100.

Glycuronic acid itself is dextrorotatory ($[\alpha]_D = +35°$), but many of its compounds are lævorotatory.[1] It reduces Fehling's solution on heating, and precipitates the metals from hot alkaline solutions of silver, mercury, and bismuth.

By treatment with bromine or nitric acid it yields saccharic acid, $C_6H_{10}O_8$, a reaction which indicates the presence of an aldehyde group and the close relation between glycuronic acid and dextrose. Saccharic acid can again be reduced to glycuronic acid by treatment with sodium amalgam, further treatment yielding gulonic acid, $C_6H_{12}O_7$, a body which does not reduce Fehling's solution. (Fischer and Piloty *Ber.*, 1891, **24,** 521). On the reduction of glycuronic acid with sodium amalgam Thierfelder (*Zeitschr. f. physiol. Chem.*, 1891, **15,** 71) obtained *d*-gulose.

When boiled with alkali hydroxide, glycuronic acid yields oxalic acid as an invariable product. Catechol and protocatechuic acid are also formed if concentrated alkali be employed for the treatment.

Glycuronic acid is distinguished from dextrose by not undergoing the alcoholic fermentation when treated with yeast, but by the action of putrefaction bacteria it splits into l-xylose and carbon dioxide. This relation of glycuronic acid to the pentoses accounts for the colour reactions which it gives with the furfural reagents.

When heated with concentrated hydrochloric acid in the presence of orcinol or ploroglucinol glycuronic acid or its conjugated derivatives give the same colouration as the pentoses; namely, with orcinol, a reddish-blue colour which turns to bluish-green and which gives absorption bands between C and D and which, when shaken out with amyl alcohol, gives a bluish-green solution which has the same absorption. With phloroglucinol a cherry-red colour is obtained and on shaking this out with amyl alcohol a red solution having absorption bands between D and E is obtained. Tollens and Rorive (*Ber.*, 1908, **41,** 1783) sug-

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[1] After taking chloral hydrate the urine contains trichlorethyl-glycuronic acid ("urochloralic acid"), a lævorotatory body which is decomposed into trichlorethyl alcohol and dextrorotatory glycuronic acid.

gest to heat the substance with naphthoresorcinol and hydrochloric acid when a dirty blue precipitate is obtained, which gives a bluish-purple solution when shaken out with ether. This ethereal solution gives two absorption bands at D. This reaction has been shown by Mandel and Neuberg (*Bioch. Zeitschrift*, 1908, **13**, 148) not to be characteristic of glycuronic acid as other aldehyde and keto-acids and other substances give a similar reaction.

Glycuronic acid forms a potassium salt which crystallises in needles. The sodium salt is similar. The zinc, cadmium, copper, silver, and calcium salts are uncrystallisable. The barium salt is amorphous and soluble in water. It is the compound employed for the isolation of glycuronic acid from urine.

With phenylhydrazine, glycuronic acid forms a brownish crystalline compound melting at 114 to 115°, but under modified conditions an amorphous, brownish-yellow substance, melting at 150° is produced. Glycuronic anhydride gives only brown globules with phenylhydrazine. On treating 1 molecule glycuronic acid with 3 molecules phenylhydrazine for 12 to 24 hours in the thermostat at 40° a glycuronic osazone, m. p. 200 to 205°, is obtained. With *p*-bromphenylhydrazine hydrochloride and sodium acetate glycuronic acid gives a *p*-bromphenyl hydrazone, which is characterized by insolubility in absolute alcohol and by its strong lævorotatory action and which is well suited for the detection of glycuronic acid. When dissolved in a mixture of alcohol and pyridine (0.2 grm. substance in 4 c.c. pyridine and 6 c.c. alcohol) it has a rotation of 7° 25′ which corresponds to $[\alpha]\frac{20}{D} = -369°$.

On distillation with hydrochloric acid, glycuronic acid is decomposed with formation of furfuraldehyde, carbon dioxide, and water. Glycuronic anhydride and urochloralic acid undergo a similar decomposition, and a trace of furfural is also obtainable by similarly treating normal urine.

F. Mann and B. Tollens (*Chem. Centr.*, 1894, **2**, 83), have proposed this reaction for the estimation of glycuronic acid and its derivatives. The carbon dioxide obtained on distilling glycuronic acid with hydrochloric acid amounted to 26.5%, whereas the yield from dextrose or lævulose was not more than 1%. The furfural yielded by glycuronic anhydride under the same treatment was 15.23% of the weight taken. Those natural compounds which readily yield glycuronic acid on treatment with dilute acids give furfural on distillation with hydro-

chloric acid, and the proportion obtained is a measure of the glycuronic acid which may be separated from such compounds. Thus euxanthic acid yielded 6.16 to 7.17% of furfural; urochloralic acid, 9.88 to 10.30%; and potassium urobutychloralate, 9.50%.

Normal urine contains 0.004 grm., glycuronic acid in 100 c.c. This is chiefly conjugated with phenol, but a small quantity is combined with indole and skatole.

Quantitative Estimation in the Urine.—100 c.c. of the urine are completely precipitated at the water-bath temperature with a saturated solution of $Ba(OH)_2$, filtered and the excess of barium hydroxide removed by passing CO_2 through the filtrate. After filtration the filtrate is evaporated to 5 to 8 c.c. volume and transferred to a hard glass tube before crystallisation begins, by the aid of 50 c.c. of a 3% hydrobromic acid solution. After thorough cooling, 2 c.c. of bromine are added and the tube sealed up and then heated for 3 hours in a water-bath. The tube is now opened and the contents filtered free from barium sulphate, the filtrate concentrated to about 20 c.c., filtered and again concentrated to 5 c.c. This is treated with a hot saturated solution of barium hydroxide until it is distinctly alkaline, evaporated to 20 c.c.; the flocculent precipitate of basic barium saccharate filtered through a small filter and washed with a saturated solution of barium hydroxide until free from halogens. Transfer the punctured filter paper and the precipitate to a flask by the aid of water and boil for some time with a saturated solution of ammonium carbonate and the addition of a little ammonia. After heating for about 1/2 hour the barium carbonate is filtered off and the filtrate evaporated in a porcelain dish on the water-bath. The syrup is taken up in water and this solution again evaporated so as to expel all the ammonium compounds. It is finally evaporated to 3 to 5 c.c. and then precipitated with a concentrated solution of silver nitrate and stirred and then allowed to stand in the dark for 2 hours. The precipitate is collected in a weighed Gooch filter, washed with 50% and then 95% alcohol, dried to constant weight in vacuum. (See Neuberg and Niemann, *Zeitschr. f. physiol. Chem.*, 1905, **44,** 127.)

Glycuronic acid occurs in the urine to a very notable extent after the administration of morphine, chloroform, chloral, butylchloral, nitrobenzene, camphor, curare, and certain other drugs. It was undoubtedly mistaken for dextrose by the older observers.

Ketonic Derivatives.

These include β-hydroxybutyric acid, aceto-acetic acid and acetone and these substances occur in the urine especially in diabetes mellitus, but also in many other diseases.

β-**Hydroxybutyric acid,** $CH_3CH(OH)CH_2COOH$, is possibly a physiological metabolic product which is normally completely oxidised, with aceto-acetic acid, $CH_3CO.CH_2COOH$, and acetone, CH_3COCH_3, as intermediary products. In diabetes and especially with lack of carbohydrates the formation of these three substances is increased or their combustion reduced so that acetone and aceto-acetic acid occur in the urine and in severe cases also β-hydroxybutyric acid (acidosis). They are probably derived from the proteins and also from the fats.

β-Hydroxybutyric acid generally forms an odourless syrup, but can also be obtained as crystals. It is readily soluble in water, alcohol, and ether. It is lævorotatory with a specific rotation of $[\alpha]_D = -24.12°$ for solutions containing 1 to 11%. β-Hydroxybutyric acid is not precipitated by basic lead acetate or by ammoniacal basic lead acetate, nor does it ferment. On boiling with water, especially in the presence of a mineral acid it decomposes into α-crotonic acid which has a melting-point of 71 to 72°, $CH_3CH(OH)CH_2COOH = H_2O + CH_3CH:CH.COOH$. On oxidation with a chromic acid mixture β-hydroxybutyric acid yields acetone.

Detection of β-Hydroxybutyric acid in the Urine.—If a urine is still lævogyrate after fermentation with yeast, the presence of β-hydroxybutyric acid is probable. A further test may be made, according to Külz, by evaporating the fermented urine to a syrup and, after the addition of an equal volume of concentrated sulphuric acid, distilling directly without cooling. α-Crotonic acid is produced, which distils over, and, after collecting in a test-tube, crystals which melt at $+72°$ separate on cooling. If no crystals are obtained, shake the distillate with ether, evaporate, and test the m. p. of the residue which has been washed with water. According to Minkowski the acid may be isolated as a silver salt.

Aceto-acetic acid, diacetic acid, $CH_3CO.CH_2COOH$, is a colourless liquid which mixes in all proportions with water, alcohol, and ether. On heating to boiling with water, especially in the presence of acids, it splits into carbon dioxide and acetone, therefore it gives the acetone reactions given below. It differs from acetone by giving the following reactions:

Gerhardt's Reaction.—Treat 10 to 15 c.c. of the urine with ferric-chloride solution until it fails to give a precipitate, filter, and add some more ferric chloride. In the presence of acetoacetic acid a wine-red colour is obtained. The colour becomes paler at the room temperature within 24 hours, but more quickly on boiling (differing from salicylic acid, phenol, sulphocyanides). A portion of the urine slightly acidified and boiled does not give this reaction on account of the decomposition of the acetoacetic acid.

Arnold and Lipliawsky's Reaction.—6 c.c. of a solution containing 1 grm. of *p*-aminoacetophenone and 2 c.c. of concentrated hydrochloric acid in 100 c.c. of water are mixed with 3 c.c. of a 1% potassium nitrite solution and then treated with an equal volume of urine. A few drops of concentrated ammonia are now added and violently shaken. A brick-red colouration is obtained. Then take 10 drops to 2 c.c. of this mixture (according to the quantity of acetoacetic acid in the urine), add 15 to 20 c.c. of HCl of sp. gr. 1.19, 3 c.c. of chloroform, and 2 to 4 drops of ferric-chloride solution and mix without shaking. In the presence of acetoacetic acid the chloroform is coloured violet or blue (otherwise only yellowish or faintly red). This reaction is more delicate than the preceding test and indicates 0.04 grm. aceto-acetic acid. Large amounts of acetone (but not the quantity occurring in urines) give this reaction according to Allard.

Bondi and Schwarz's Reaction.—5 c.c. of the urine is titrated drop by drop with iodine-potassium iodide solution until the colour is orange-red. Then warm gently and when the orange-red colour has disappeared add the iodine solution again until the colour remains permanent on warming. Then boil, when the irritating vapours of iodo-acetone will attack the eyes. Acetone does not give this reaction.

Acetone, CH_3COCH_3, which occurs to a slight extent in normal urine, but in much greater amounts in diabetes mellitus and many other diseases, gives a peculiar odour to this fluid. It also gives an odour to the expired air similar to apples. Acetone boils at 56.3°, mixes with water, alcohol, and ether and may be detected by the following reactions (see also Vol. I.

Lieben's Iodoform Test.—When an aqueous solution of acetone is treated with alkali and then with some iodo-potassium-iodide solution and gently warmed, a yellow precipitate of iodoform is formed, which is known by its odour and by the appearance of the crystals (six-sided plates or stars) under the microscope. This reaction is very delicate,

but it is not exclusively characteristic of acetone. Gunning's modification of the iodoform test consists in using an alcoholic solution of iodine and ammonia instead of the iodine dissolved in potassium iodide and alkali hydrate. In this case, besides iodoform, a black precipitate of iodide of nitrogen is formed, but this gradually disappears on standing, leaving the iodoform visible. This modification has the advantage that it does not give any iodoform with alcohol or aldehyde. On the other hand, it is not quite so delicate, but still it detects 0.01 mgrm. of acetone in 1 c.c.

Reynold's mercuric-oxide test is based on the power of acetone to dissolve freshly precipitated HgO. A mercuric-chloride solution is precipitated by alcoholic potassium hydroxide. To this add the liquid to be tested, shake well and filter. In the presence of acetone the filtrate contains mercury, which may be detected by ammonium sulphide. This test has about the same delicacy as Gunning's test. Aldehydes also dissolve appreciable quantities of mercuric oxide.

Legal's Sodium Nitroprusside Test.—If an acetone solution is treated with a few drops of a freshly prepared sodium-nitro-prusside solution and then with potassium hydroxide or sodium hydroxide solution, the liquid is coloured ruby-red. Creatinine gives the same colour; but if the mixture is saturated with acetic acid, the colour becomes carmine or purplish red in the presence of acetone, but yellow and then gradually green and blue in the presence of creatinine. With this test paracresol responds with a reddish-yellow colour, which becomes light pink when acidified with acetic acid and cannot be mistaken for acetone. Rothera (*J. Physiol.*, 1908, **37,** 49) has suggested a modification which is more delicate by using ammonium salts and ammonia.

Penzoldt's indigo test depends on the fact that orthonitrobenzaldehyde in alkaline solution with acetone yields indigo. A warm saturated and then cooled solution of the aldehyde is treated with the liquid to be tested for acetone and next with sodium hydroxide. In the presence of acetone the liquid first becomes yellow, then green, and lastly indigo separates; and this may be dissolved with a blue colour by shaking with chloroform; 1.6 mgrm. acetone can be detected by this test.

Bela v. Bitto's reaction is based on the fact that on adding a solution of *m*-dinitrobenzene made alkaline with potassium hydroxide to acetone, a violet-red colour is produced which becomes cherry-red on acidifying

with an organic acid or metaphosphoric acid. Aldehyde gives a similar violet-red colour which becomes yellowish-red on acidification. Creatinine does not give this reaction. Frommer has suggested the following method for detecting acetone: Treat 10 c.c. of the urine with 1 grm. potassium hydroxide and add 10 to 20 drops of an alkaline solution of salicyl-aldehyde. On warming a purple-red colouration is obtained in the presence of acetone.

The **quanity of β-hydroxybutyric acid** can be estimated by completely extracting the acid by ether and determining the specific rotation. The following methods are those usually employed.

Shaffer's Method.—25 to 250 c.c., depending upon the amount of β-hydroxybutyric acid present, of the urine are placed in a 500 c.c. flask and precipitated by an excess of basic lead acetate and 10 c.c. ammonia. The mixture is diluted to 500 c.c., shaken and filtered. 200 c.c. of the filtrate is placed in an 800 c.c. Kjeldahl digestion flask, 300 to 400 c.c. water, 15 c.c. concentrated sulphuric acid and a little talcum added and the mixture distilled until 200 to 250 c.c. of the distillate has been collected. This distillate (A) contains the acetone (both preformed and that produced from the aceto-acetic acid) and volatile fatty acids. It is treated with 5 c.c. of 10% potassium hydroxide solution and distilled again to remove the volatile fatty acids. This second distillate (A₂) is now titrated with standard iodine and thiosulphate. The residue obtained from distillate (A) is now distilled with 400 to 600 c.c. of a 0.1 to 0.5% potassium dichromate solution,[1] which is added by means of a dropping tube, during the process of the distillation. When about 500 c.c. of the distillate (B) has collected add 20 c.c. of a 3% solution of hydrogen peroxide and a few c.c. of a potassium hydroxide and re-distill. Collect about 300 c.c. distillate (B₂) and titrate with iodine and thiosulphate. Shaffer recommends the use of solutions of thiosulphate and iodine which are a trifle stronger than $N/10$—*i.e.*, 1.034 $N/10$. Each c.c. of an iodine solution of this strength is equivalent to 1 mgrm. of acetone or to 1.794 mgrm. of β-hydroxybutyric acid. The titration of distillate (A₂) gives the amount of acetone (either preformed or produced from aceto-acetic acid) and the titration of distillate (B₂) gives the amount of acetone derived from the β-hydroxybutyric acid by oxidation.

Bergell's Method (Zeit. physiol. Chem., 1901, 33, 310).—Make 100

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[1] In the presence of large amounts of sugar or when large volumes of the urine are used it may be necessary to use more dichromate.
[2] The sugar can be removed, if present, by fermentation with yeast.

to 300 c.c. of sugar-free[2] urine alkaline with sodium carbonate, evaporate to a syrup, cool, and rub the syrup up with concentrated phosphoric acid, keeping the mixture cool, add 20 to 30 grm. finely pulverised anhydrous copper sulphate and 20 to 25 grm. of fine sand. The mass is thoroughly mixed, placed in an extraction thimble and extracted in a Soxhlet extraction apparatus, with ether. The ether is evaporated, the residue dissolved in about 25 c.c. of water, decolourised with animal charcoal if necessary and the β-hydroxybutyric acid estimated quantitatively by polarisation. Magnus-Levy (*Ergeb. d. immeren Med. u. Kinderheil.*, 1908) extracts the β-hydroxybutyric acid from the urine (100 to 1,000 c.c. according to the amount of acid present) after adding ammonium sulphate and sulphuric acid, in a Zelmannowitz extraction apparatus, and evaporates the ethereal extract spontaneously to dryness. The residue is completely extracted with water and the quantity of β-hydroxybutyric acid determined by the polariscope after filtering.

The quantitative estimation of acetone (also that formed from the acetoacetic acid) is done by distilling the urine after the addition of acetic acid or a little sulphuric acid. The quantity of acetone in the distillate can be determined, according to the Huppert-Messinger method, by converting it into iodoform by means of potassium iodide and then titrating the quantity of iodine used in the formation of the iodoform. The precipitation of the acetone as p-nitrophenylhydrazone by means of p-nitrophenylhydrazine in acetic acid solution can also be used for determining the acetone in the distillate (v. Ekenstein and Blanksma and Möller).

Aromatic Hydroxyacids.—In the putrefaction of proteins in the intestine p-hydroxyphenyl-acetic acid, $C_6H_4(OH)CH_2COOH$ and p-hydroxyphenyl-propionic acid, $C_6H_4(OH)$ CH_2CH_2COOH, are formed from tyrosine and these pass into the urine. The quantity is increased under the same conditions as the phenols. Both of these acids are soluble in water and ether and give a beautiful red colouration on being warmed with Millon's reagent. The first one melts at 148° and the second at 125°.

To detect the presence of these hydroxyacids proceed as follows: Warm the urine for a while on the water-bath with hydrochloric acid in order to drive off the volatile phenols. After cooling, shake three times with ether and then shake the ethereal extracts with dilute sodium hydroxide solution, which dissolves the hydroxyacids, while the phenols remain in the ether. The alkaline solution of the hydroxy-

acids is now faintly acidified with sulphuric acid, shaken again with
ether, the ether removed and allowed to evaporate. The residue
is dissolved in water and the solution tested with Millon's reagent.
The two hydroxyacids are best differentiated by their melting-points.

Besides these two hydroxyacids we sometimes have other hydroxy-
acids in urines; namely, homogentisic acid, uroleucic acid, hydroxy-
mandelic acid, hydroxyparacoumaric acid, and kynurenic acid (only
in dogs' urine). *Homogentisic acid*, dihydroxylphenylacetic acid,

$$C_6H_3{<}{\overset{\displaystyle OH}{\underset{\displaystyle CH_2COOH,}{OH}}}$$
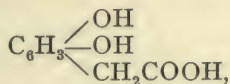
was discovered by Marshall and isolated from the urine by Wolkow
and Baumann in a case of *alcaptonuria*. The formation of
homogentisic acid is due to an anomaly in the metabolism of tyrosine
and phenylalanine in the body of alcaptonurics, where the demoli-
tion of these bodies stops at this point.

Homogentisic acid crystallises with 1 H_2O in large, transparent
prismatic crystals, which become non-transparent at the temperature
of the room with the loss of water of crystallisation. They melt
at 146.5 to 147°. They are soluble in water, alcohol, and ether,
but nearly insoluble in chloroform and benzene. Homogentisic acid
is optically inactive and non-fermentable. Its aqueous solution has
the properties of so-called alcaptonuric urine. It becomes greenish-
brown from the surface downward on the addition of very little sodium
hydroxide or ammonia with access of oxygen, and on shaking it quickly
becomes dark brown or black. It reduces alkaline copper solutions
on slightly warming, and ammoniacal silver solutions immediately
in the cold. It does not reduce akaline bismuth solutions. It gives
a lemon-coloured precipitate with Millon's reagent, which becomes
light brick-red on warming. Ferric chloride gives to the solution a
blue colour which soon disappears. On boiling with concentrated
ferric chloride solution an odour of quinone develops. With benzoyl
chloride and sodium hydroxide in the presence of ammonia we obtain
the amide of dibenzoylhomogentisic acid, m. p. 204°, which can be
used in the isolation of the acid from the urine, and also for its detec-
tion (Orton and Garrod, *J. Physiol.*, 1901, **27,** 89). Among the
salts of this acid must be mentioned the lead salt containing water of
crystallisation and 34.79% Pb. This salt melts at 214 to 215°.

In order to prepare the acid, heat the urine to boiling, add 5 grm. of lead acetate for every 100 c.c., filter as soon as the lead acetate has dissolved, and allow the filtrate to stand in a cool place for 24 hours until it crystallises (Garrod). The dried, powdered lead salt is suspended in ether and decomposed by H_2S. After the spontaneous evaporation of the ether the acid is obtained in nearly colourless crystals (Orton and Garrod).

Uroleucic acid, $C_9H_{10}O_5$, is, according to Huppert, probably a di-hydroxyphenyl-lactic acid, $C_6H_3(OH)_2CH_2CH(OH)COOH$. This acid was first prepared by Kirk from the urine of children with alcaptonuria, which also contained homogentisic acid. It melts at 130 to 133°. Otherwise in regard to its behaviour with alkalies, with access of air, and also with alkaline copper solutions and ammoniacal silver solutions and also Millon's reagent, it is similar to homogentisic acid.

Hydroxymandelic acid, $C_8H_8O_4$, *p*-hydroxyphenylglycollic acid, $HO.C_6H_4CH(OH)COOH$, is found in the urine in acute atrophy of the liver. The acid crystallises in silky needles. It melts at 162°, dissolves readily in hot water, less in cold water, and readily in alcohol and ether, but not in hot benzene. It is precipitated by basic lead acetate, but not by lead acetate.

Kynurenic acid (4-hydroxy-3-quinoline-carboxylic acid), $C_{10}H_7ON_2$
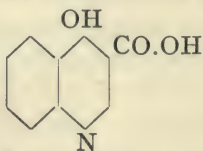
has only been found thus far in dogs' urine, but not always; its quantity is increased by meat feeding. It does not occur in the urine of cats. Ellinger has been able to show positively that tryptophane is the mother-substance of this acid.

The acid is crystalline, does not dissolve in cold water, rather well in hot alcohol, and yields a barium salt which crystallises in triangular, colourless plates. On heating it melts and decomposes into CO_2 and kynurin. On evaporation to dryness on the water-bath with hydrochloric acid and potassium chlorate a reddish residue is obtained which becomes first brownish-green and then emerald-green on adding ammonia (Jaffé's reaction).

Oxyproteic Acids.—A series of nitrogenous sulphur-containing acids have been isolated by Bondzinski, Dombrowski and Panek (*Zeit. f. physiol. Chem.*, 1905, **46**, 83) from human urine which are of interest because of their relationship to the proteins. They are antoxyproteic acid, containing 24.4% N and 0.61% S, oxyproteic acid with 18.08% N and 1.12% S, alloxyproteic acid with 13.55% N and 2.19% S.

Antoxyproteic acid is soluble in water, dextrorotatory and is precipitated from its concentrated solution by phosphotungstic acid. It does not give the protein colour reactions, but does give Ehrlich's diazo-reaction. The salts of the alkalies, barium, calcium, and silver are soluble in water, while the barium and especially the silver salt is difficultly soluble in alcohol. The free acid and its salts are precipitated by mercuric nitrate or acetate even in strongly acetic acid solution. Basic lead acetate does not precipitate the pure acid.

Oxyproteic acid does not give the Ehrlich diazo reaction nor the xanthoprotein or the biuret reactions. It gives a faint Millon reaction and it is not precipitated by phosphotungstic acid. Oxyproteic acid is precipitated by mercuric nitrate or acetate in neutral reaction, but not by basic lead acetate. The salts are more readily soluble in water than the corresponding salts of the antoxyproteic acid.

Alloxyproteic acid is soluble in water, does not give the biuret reaction or Ehrlich's diazo reaction and is not precipitated by phosphotungstic acid. It differs from the other two acids by being precipitated by basic lead acetate, and its salts are less soluble in alcohol than the other salts.

Uroferric acid is an acid isolated by Thiele (*Zeitschr. f. physiol. Chem.*, **37**, 251), which contains 3.46% S and has the formula $C_{85}H_{56}O_{19}N_8S$. This acid is readily soluble in water, saturated ammonium sulphate solution and methyl alcohol. It is soluble with difficulty in absolute alcohol and insoluble in benzene, chloroform ether and glacial acetic acid. It does not respond to either the biuret reaction or to Millon or Adamkiewicz's reaction. It is precipitated by mercuric nitrate and sulphate and also by phosphotungstic acid. Uroferric acid has a rotation $[\alpha]_D = -32.5°$ at 18°. The existence of this acid is disputed by Bondzynski, Dombrowski and Panek.

For the quantitative estimation of the total oxyproteic acids in urine we refer to the complicated methods suggested by Ginsberg (Hofmeister's *Beiträge*, 1907, **10**, 411) and Gawinski (*Zeitschr. f. physiol. Chem.*, 1909, **58**, 454).

Amino Acids.—Normal human urine contains small but varying amounts of amino acids, while under pathological conditions the amount may be considerable. The amino acid fraction seems to be raised in starvation and in certain pathological conditions. By means of the naphthalensulphochloride method the presence of glycocoll has been demonstrated in normal human urine and this glycocoll does not exist free but in a combination, which is split by alkali. Various methods for the estimation of the total amino-acid nitrogen in urine have been suggested, but the method of Henriques (*Zeitschr. f. physiol. Chem.*, 1909, **60**, 1) based upon Sörensen's (*Bioch. Zeitschr.*, 1908, **7**, 45), formol titration and the method proposed by Van Slyke (*Proc. Soc. for Exp. Biol. and Med.*, 1909, **7**, 46) are to be recommended (see also pp. 262–270, and Vol. 8).

Henriques' Method.—This titration method is based upon the fact that if a neutral solution of formaldehyde is added to an amino-acid solution the amino groups form a methylene combination with the formaldehyde and make possible the determination of the carboxyl groups present by simple titration. The following formula explains the process:

$$R\ CH(NH_2)COOH + HCOH = R\ CH(N:CH)COOH + H_2O$$
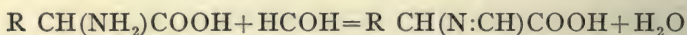
50 c.c. of the urine is placed in a 100 c.c. graduated flask, 1 c.c. of a 0.5% phenolphthaleïn solution and 2 grm. solid barium chloride added. After solution of the barium chloride, add a saturated solution of barium hydroxide until it is alkaline and then add 5 c.c. more of the same solution (to remove the phosphates). Now fill to 100 c.c. with water, shake well, allow to stand for 15 minutes and filter through a dry filter, collecting 80 c.c. of the filtrate (corresponding to 40 c.c. of the urine). This is placed in a 100 c.c. flask and the liquid neutralised with $N/5$ hydrochloric acid using litmus as indicator and then filled up to 100 c.c. with water. The ammonia is estimated in 40 c.c. (= 16 c.c. urine) by the Folin-Shaffer method or any other method and the other portion is used for the formaldehyde titration, according to Sörensen's suggestion.

The following solutions are necessary: 1. A solution of 0.5 grm. phenolphthaleïn in 50 c.c. alcohol and 50 c.c. water. 2. A freshly prepared formaldehyde mixture which consists of 50 c.c. commercial formaldehyde (30 to 40%) to which 1 c.c. phenolphthaleïn solution is added and then $N/5$ barium hydroxide solution added until the solu-

tion has a faint pink colour. As a control solution 20 c.c. boiled distilled water are placed in a flask, 10 c.c. of the above formaldehyde solution added and then about 5 c.c. of $N/5$ barium hydroxide solution added from a burette and then retitrated with $N/5$ HCl, added drop by drop, until the colour of the solution is just faintly pink (first stage). Then 1 drop of the barium hydroxide solution is added when a pronounced pink colour should be the result (second stage). The urine is now titrated to this same colour by taking 20 c.c. of the solution and adding 10 c.c. of the formaldehyde mixture and $N/5$ barium hydroxide solution added to a deep red colour, then retitrated with $N/5$ hydrochloric acid until the colour is fainter than the control solution and finally barium hydroxide added drop by drop until the colour is same as the control solution. The number of c.c. of $N/5$ barium hydroxide actually used to neutralise the solution when multiplied by 2.8 gives the mgrm. of nitrogen as amino-acid nitrogen after subtracting the ammonia nitrogen, as specially determined. $N/5$ sodium hydroxide can be used instead of the $N/5$ barium hydroxide. Urea, creatine, creatinine and hippuric acid do not influence the results. If the fomaldehyde titration differs before and after boiling with strong hydrochloric acid, it indicates the presence of polypeptides.

Acids of Bile.

The bile contains certain conjugated acids which are strictly peculiar to that secretion. They occur as sodium salts, and are not found in the pancreatic juice, or in other normal animal secretions.

Human bile is a reddish, reddish-brown, or dirty green liquid, having an odour like that of musk, a very bitter taste, and a faintly alkaline reaction. The sp. gr. averages about 1.020. In its original condition, bile rapidly putrefies, but if the secretion be diluted, acidified with acetic acid, and filtered from the precipitated mucin, etc., it may be readily preserved.

Bile is a secretion of a very variable character, and its collection in a normal state is attended with peculiar difficulties. The following analyses show the general composition of the human liver bile in 1000 parts:

Solids...	25.200	35.260	25.400
Water..	974.800	964.740	974.600
Mucin and pigments................................	5.290	4.290	5.150
Bile salts...	9.310	18.240	9.040
Taurocholate......................................	3.034	02.079	2.180
Glycocholate......................................	6.276	16.161	6.860
Fatty acids besides soaps.........................	1.230	1.360	1.010
Cholesterol.......................................	0.630	1.600	1.500
Lecithin }	0.220	00.574	0.650
Fat }		0.956	0.610
Soluble salts.....................................	8.070	6.760	7.250
Insoluble salts...................................	0.250	0.490	0.210

The gall-bladder bile is richer in solids, ranging from 8 to 18%.

A complex and concentrated solution such as bile is very apt to form deposits under abnormal conditions. Hence arise the well-known concretions called biliary calculi and gall-stones.[1]

The two chief acids of bile are glycocholic and taurocholic acid. The former of these is the more abundant in the human and ox-bile, in the proportion of fully 3 to 1; but is replaced by taurocholic acid in the bile of the dog and carnivora generally. Other bile-acids of less frequent occurrence and abundance are also met with (page 416). This follows from the fact that there are several cholic acids.

For the preparation of the mixed sodium salts of the bile-acids, ox-bile should be mixed with washed sand, and evaporated at 100° till the residue can be powdered. The product is then extracted with boiling absolute alcohol, which dissolves the salts of the bile-acids, while leaving pigment, mucin, and a portion of the inorganic salts undissolved. The green alcoholic solution is filtered and boiled with animal charcoal till colourless,[2] when it is again filtered, the residue

[1] **Biliary Calculi.**—Under this denomination are comprised all those concretions which are formed in the bile. They are found in all parts of the biliary apparatus, occurring most frequently in the gall-bladder or gall-ducts, but sometimes in the intestinal canal. Their size varies from very small granules to (occasionally) that of a pigeon's egg. The form is generally oval, but when several calculi occur together in the gall-bladder, facets are generally formed by their mutual attrition. The colour of biliary calculi ranges from nearly white to yellow, brown, and dark green. Gall-stones are generally brittle, and can be readily reduced to a powder having a greasy feel.

Gall-stones usually contain cholesterol as their leading constituent, calcium carbonate and bile-pigments being also present in very variable proportion. Fats, silica, uric acid, and compounds of iron, zinc, copper, and manganese have been observed as occasional constituents. Sometimes the bile-pigments preponderate, occasionally amounting to 60% of the calculus. Besides bilirubin and biliverdin, there have been found in gall-stones: biliprasin, $C_{16}H_{22}O_6N_2$, bilifuscin, $C_{16}H_{10}O_4N_2$, bilicyanin, bilihumin, etc. The bilirubin exists as a calcium salt, $Ca(C_{16}H_{17}O_3N_2)_2(?)$, which circumstance prevents the solution of the colouring matter in chloroform unless the stone be previously treated with acid. On boiling powdered gall-stones with alcohol or ether, cholesterol is almost the only constituent dissolved. Dilute hydrochloric acid will subsequently dissolve the calcium, whether existing as carbonate or as the bilirubin compound, and chloroform will then dissolve the bilirubin and bilifuscin. Subsequent boiling with alcohol will dissolve biliverdin and biliprasin, while bilihumin remains insoluble.

Biliary calculi are usually saturated with bile, which has desiccated after removal from the organism. The nucleus generally consists of mucus.

[2] An alternative plan is to mix the original bile into a paste with animal charcoal, dry the mixture at 100°, and exhaust it with boiling absolute alcohol.

taken up in a minimum quantity of absolute alcohol, and ether added until a permanent turbidity is produced. On standing for a few hours, the mixed sodium salts of glycocholic and taurocholic acids will be deposited as a white, semi-crystalline mass known as "Plattner's crystals," which should be pressed between blotting paper and dried.

From the sodium salts prepared as above, free glycocholic acid may be readily obtained by dissolving the crystals in a little water, adding ether and then dilute sulphuric acid as long as a precipitate is produced. On stirring, glycocholic acid separates as a crystalline mass of shining needles, while the very soluble taurocholic acid remains in solution.

Both glycocholic and taurocholic acid generally undergo hydrolysis under the influence of dilute acids or alkalies. In each case one of the products of the reaction is cholalic acid. In the case of glycocholic acid, the second product is glycocoll. The following equations express the reactions:

a.
$$C_{26}H_{43}O_6N + H_2O = C_{24}H_{40}O_5 + C_2H_5O_2N$$
Glycocholic acid.   Cholalic acid.   Glycocoll.

b.
$$C_{26}H_{45}O_7NS + H_2O = C_{24}H_{40}O_5 + C_2H_7O_3NS$$
Taurocholic acid.   Cholalic acid.   Taurine.
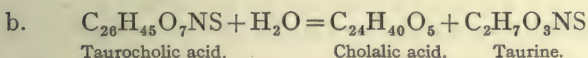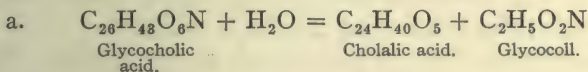
These changes occur naturally in the intestine. In a state of health, by far the larger proportion of the products is re-absorbed, and passes back into the liver.

Glycocholic acid, $C_{26}H_{43}O_6N$, or $C_{23}H_{39}O_3.CO.NH.CH_2COOH$. This acid was first described by Gmelin, in 1826, under the name of cholic acid. It occurs as a sodium salt in human and ox-bile to the extent of 3 to 5%, together with more or less of the analogous taurocholic acid. The bile of the herbivora generally contains glycocholic acid, but that of the carnivora contains taurocholic acid with mere traces of glycocholic acid.

Glycocholic acid may be readily prepared by the process described on page 413. By pressing the crystals, and recrystallising the acid from hot water, it is obtained perfectly pure. It has also been prepared synthetically by Bondi and Müller (*Zeitschr. f. physiol. Chem.*, 1906, **47,** 499) by the action of nitrous acid upon cholalic acid hydrazide and then the conjugation of glycocoll with the azide thus obtained.

Marshall prepared glycocholic acid by treating fresh bile with a little

hydrochloric acid, and filtering from the precipitate of mucin, etc. 100 volumes of the filtrate are then treated with 5 volumes of ether and the mixture shaken and allowed to stand for some hours.[1] The crystals of glycocholic acid which form are then filtered off, washed with water containing ether and hydrochloric acid, dried in the air, and recrystallised from hot water.

Glycocholic acid forms fine glistening needles, or prisms, which taste at first sweet and afterward bitter. The m. p. varies between 132 and 152°, depending upon the method of preparation. Emich found the melting-point for the acid crystallised from water to be 132 to 134°.

Glycocholic acid is soluble in about 300 parts of boiling water, and is very soluble in alcohol; but is very slightly soluble in ether, and practically insoluble in chloroform and benzene. Glycocholic acid forms salts which are extremely soluble both in water and in alcohol, but very slightly soluble or insoluble in ether. An alcoholic solution of the free acid has a rotation of $[\alpha]_D = +29.0°$ and the rotation of the sodium salt is $+25.7°$.

Sodium glycocholate, $NaC_{26}H_{42}O_6N$, forms stellate needles. Potassium glycocholate occurs in the bile of certain fishes.

When dissolved in warm concentrated sulphuric acid (or, according to Strecker, by simply heating above 100°), glycocholic acid loses the elements of water, and is converted into glycocholonic acid, $C_{26}H_{41}O_5N$, a body forming an insoluble barium salt, but possessing nearly the same optical activity as the parent acid.

As stated on page 411, when boiled with dilute acids or alkalies, glycocholic acid undergoes hydrolysis with formation of cholalic acid and glycocoll. The reaction is analogous to the formation of benzoic acid and glycocoll from hippuric acid.[2]

Glycocholeic acid, $C_{26}H_{43}O_5N$, or $C_{27}H_{45}O_5N$, is a second glycocholic acid isolated from ox-bile by Wahlgren (*Zeitschr. f. physiol. Chem.*, 1902, **36,** 556), and which also occurs in human bile. It crystallises in short prisms or needles and is insoluble in cold water and slightly soluble in boiling water. It is soluble in alcohol but not

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[1] F. Emich (Monh. f. Chem., 1882, **3,** 325) recommends the use of benzene instead of ether in this process, and states that bile which gives no precipitate with ether readily gives crystals when benzene is employed.

[2] According to F. Emich, when a saturated aqueous solution of glycocholic acid is boiled or many hours, about 22% is converted into *p*-glycocholic acid, an intensely bitter substance melting at 183°, and nearly insoluble in water. It seems probable that under the treatment employed the glycocholic acid suffers hydrolysis, with formation of cholic acid of one of its decomposition-products, which is the substance obtained by Emich.

very soluble in ether or acetone, while it is insoluble in benzene and chloroform. Glycocholeic acid, m. p. 175 to 176°, gives a brownish-yellow solution with a strong green fluorescence with concentrated sulphuric acid. On hydrolytic cleavage it yields glycocoll and choleic acid. The alkali salts are soluble in water and are more readily precipitated by neutral salts (NaCl) than the glycocholates.

The principle of the preparation of the pure glycocholic acids consists in treating a 2 to 3% solution of bile free from mucus, when rich in glycocholic acid, with ether and then with 2% hydrochloric acid. If the bile is not directly precipitable with hydrochloric acid (bile relatively poor in glycocholic acid), then first precipitate the chief mass of the glycocholic acid with ferric chloride, or better with lead acetate, decompose the precipitate with sodium hydroxide and treat the 2% solution, as above stated, with ether and hydrochloric acid. The crystalline and washed mass is boiled with water and on cooling, glycocholic acid crystallises out and then this is recrystallised from water or from alcohol by the addition of water. The residues that remains after boiling in water (paraglycocholic acid and glycocholeic acid) are converted into barium salts and after a complicated process the glycocholeic acid is obtained.

Taurocholic Acid.—$C_{25}H_{45}O_7NS$, or $C_{22}H_{39}O_3.CO\ NH\ CH_2CH_2$-$SO_2OH$. This acid is a constituent of the bile of all carnivora, and exists in human bile together with glycocholic acid. The preparation of pure taurocholic acid from human or ox-bile is difficult, since the portion of the glycocholic acid which remains in solution with the more readily soluble taurocholic acid is very troublesome to separate therefrom. Hence it is preferable to prepare taurocholic acid from dog's bile, which should be treated by the process described on page 410 for the preparation of "Plattner's crystals." The sodium salt is dissolved in water, and the taurocholic acid precipitated as a lead salt by addition of ammonia and basic lead acetate. The precipitate is filtered off, washed, suspended in alcohol, and decomposed by hydrogen sulphide. The filtered liquid is concentrated and treated with excess of ether, when taurocholic acid is precipitated as a syrupy mass, which may become partly crystalline on standing.

The amount of taurocholic acid present in bile may be estimated without isolating it by calculation from the amount of sulphur contained in the alcoholic extract of the bile, since no other sulphuretted substance passes into the alcoholic solution. For this purpose, the

dried alcoholic extract from a known quantity of bile is evaporated to dryness on a water-bath with fuming nitric acid, by which treatment the sulphur is converted into sulphuric acid. The residue is taken up with water, and the solution precipitated with barium chloride. One part of $BaSO_4$ corresponds to 2.16 parts of taurocholic acid.

Taurocholic acid forms deliquescent silky needles, very soluble in water and in alcohol, but insoluble in ether, benzene, and acetone. The same ready solubility characterises the salts of taurocholic acid except the precipitate produced by the basic lead acetate in presence of ammonia, which is insoluble in water but soluble in boiling alcohol. Taurocholic acid has the ability of holding the insoluble glycocholic acid in solution.

Taurocholic acid and its salts are dextrorotatory, the value of $[\alpha]_D$ for the solution of the sodium salt in alcohol being $+24.5°$. On heating, taurocholic acid contracts at 140°, begins to decompose at 160°, and melts at 180° to a brown liquid.

By boiling with dilute acids or alkalies, taurocholic acid is hydrolysed with formation of cholalic acid and taurine (page 411). The same decomposition occurs on merely boiling an aqueous solution of taurocholic acid, and takes place naturally in the intestine. This behaviour accounts for the absence of unchanged taurocholic acid from the urine, in which it is represented by taurine and taurocarbamic acid.

Taurocholic acid possesses the power of completely precipitating albumins and globulins from their solutions, but it does not precipitate peptones. It is stated to possess powerful antiseptic properties.

Taurocholeic acid, $C_{26}H_{45}O_6NS$, or $C_{27}H_{47}O_6NS$, is found in dog-bile and ox-bile. It is amorphous, readily soluble in water and in alcohol, but insoluble in ether, acetone, chloroform, and benzene. The soluble alkali salts can be salted out by sodium chloride. On cleavage taurocholeic acid yields taurine and choleic acid.

Hyoglycocholic acid, $C_{27}H_{43}O_5N$, the crystalline glycocholic acid from pig's bile, is characterised by insolubility in water, intense bitter taste and by being precipitated by calcium, barium and magnesium chlorides.

Cheno-taurocholic acid, $C_{29}H_{47}O_6NS$, is the chief taurocholic acid of goose-bile.

Cholalic acid, *or cholic acid*, $C_{24}H_{40}O_5$, or $C_{20}H_{31}$ $\begin{cases} CO.OH \\ (CH_2.OH)_2. \\ CH.OH \end{cases}$

Cholalic acid is the acid product of the hydrolysis both of glycocholic

acid and taurocholic acid, the former yielding glycine and the latter taurine as the basic product of the decomposition (page 411).

Cholalic acid occurs in the small and large intestines as a product of the decomposition of the bile-acids. It is also present in the fæces of men and the lower animals.

For the preparation of cholalic acid, ox-bile should be boiled for 24 hours, in a flask fitted with a reflux condenser, with as much caustic baryta as it will take into solution. The liquid is filtered while still hot, and the filtrate concentrated until it yields a copious crop of crystals of barium cholalate. The salt is recrystallised from boiling water, decomposed by hydrochloric acid, and the free cholic acid crystallised from a small volume of boiling alcohol. Or the acid may be dissolved in sodium hydroxide containing a little ether, and the solution acidulated with hydrochloric acid. The crystals which form after a time are separated and treated with ether, which is poured off after half an hour, and the residue dissolved in boiling alcohol. Water is gradually added to this solution till a permanent precipitate appears, when cholalic acid will crystallise out on cooling.[1]

Cholalic acid crystallises as rhombic plates or prisms with 1 molecule of water of crystallisation and in large rhombic tetrahedra or octahedra with 1 molecule alcohol of crystallisation. The crystals are very sparingly soluble in water, requiring 750 parts even of the boiling solvent, but are soluble in alcohol and soluble with difficulty in ether. The alcohol of crystallisation is driven off by heating to 100 to 120° for a long time and the acid free from water and alcohol melts at about 195°. The specific rotation of the crystalline acid is $[\alpha]_D = +31.55°$ and $[\alpha]_D + 37.02°$ for the anhydrous acid. Cholalic acid gives a blue compound with iodine and if the finely powdered acid is added to 25% hydrochloric acid at the ordinary temperature a beautiful violet-blue colouration gradually appears and in allowing to stand it gradually changes to green and yellow. The blue solution shows an absorption band at D.

[1] The same method may be employed for the estimation of the cholalic acid obtainable from bile, but for this purpose Lassar-Cohn (*Ber.*, 1893, **26**, 146) recommends the following process: 20 c.c. of the bile is mixed with 2 grm. of sodium hydroxide, the liquid boiled for 24 hours, saturated with carbon dioxide, and evaporated to dryness at 100°. The residue is boiled with nearly absolute alcohol, until free from the salts of organic acids which are only sparingly soluble in water, and the solution, after dilution with 4 measures of water, is precipitated by 0.5 grm. of barium chloride in dilute solution. The filtered liquid is acidified with hydrochloric acid, and shaken with ether. This, in presence of alcohol, readily extracts the cholic acid, which is obtained on evaporating the ethereo-alcoholic solution. Lassar-Cohn obtained the following percentage of acids from a sample of ox-bile treated in the above manner: Cholic acid, 4.790; choleic acid, 0.085; myristic acid, 0.004; stearic and palmitic acids, 0.146; resinous acids, 0.120; and loss, 0.050%.

The alkali salts are readily soluble in water and less soluble in alcohol. Barium cholalate forms fine silky needles (often radiated) very soluble in boiling water and in alcohol. The sodium and barium salts of the cholalic acid isolated from human bile are less soluble than the corresponding compounds prepared from ox-bile. The difference has been attributed to the non-identity of the cholalic acid from the two sources, but it appears to be really due to contamination of the cholalic acid from human bile with a small quantity of the analogous fellic acid. The cholalic acids from the bile of several other animals also exhibit certain differences, which in some cases at least are due to the presence of associates of ordinary cholalic acid. Thus:

Choleic acid, $C_{25}H_{42}O_4$, is obtained in small quantity, together with cholalic acid, when the latter is prepared from ox-bile. It is soluble at 20° in 22,000 parts of water, in 750 of ether, in 14 parts of absolute alcohol, and in 25 parts of alcohol of 75%. Barium choleate dissolves in 1,200 parts of cold water, the solubility rapidly increasing with the temperature. The specific rotation of the alcoholic solution with a concentration of 2.49% is $[\alpha]_D = +48.87°$. The barium salt which crystallises in spherical aggregations of needles from hot alcohol is less soluble in water than the corresponding cholic acid salt.

Desoxycholic acid, $C_{24}H_{40}O_4$, was obtained, together with cholalic acid, from bile which had been allowed to putrefy for some time. Desoxycholic acid differs from cholalic acid in its intense bitter taste, its ready solubility in alcohol, and in its sparing solubility in glacial acetic acid and it does not give any blue iodine compound nor does it give the colour reaction with hydrochloric acid. Its barium salt is soluble with difficulty in cold water, but dissolves in boiling alcohol. The acid crystallised from a mixture of alcohol and ether melts at 153 to 155°.

Fellic acid, $C_{23}H_{40}O_4$, occurs with cholic acid in human bile. It has a bitter taste, m. p. 120°, and forms very sparingly soluble barium and magnesium salts. Fellic acid gives a red colour but not a violet colour with Pettenkofer's test (C. Schotten, *Zeits. physiol. Chem.*, 1887, **11,** 268).

Hyocholic acid, $C_{25}H_{40}O_4$, and *chenocholic acid*, $C_{27}H_{44}O_4$, are obtained by the hydrolysis of the conjugated acids of the bile of the pig and the goose respectively

Lithofellic acid, $C_{20}H_{36}O_4$, is the cholalic acid obtained from the

oriental bezoar stones, which is insoluble in water but readily soluble in alcohol.

Special cholalic acids occur in the bile of various animals such as the polar bear, walrus, sea dog, etc.

Choloidic acid, $C_{24}H_{38}O_4$, and *dyslysin*, $C_{24}H_{36}O_3$, are products of the dehydration of cholalic acids by boiling for some time with hydrochloric or sulphuric acid, or by exposure to a temperature of 200°. Dyslysin occurs in fæces, and is an amorphous substance, soluble in a large quantity of ether, and is dissolved by solutions of cholalic acid and its salts. On boiling with alkali hydroxide, it is retransformed to the corresponding cholalic acids. The various modifications of the cholalic acid obtainable from different sources are each said to yield their own variety of dyslysin.

The colour-reaction for bile acids known as Pettenkofer's test is described on page 420.

The following is a tabular scheme for the separation of bile-acids:

SCHEME FOR THE SEPARATION OF BILE ACIDS.

Dissolve the sodium salts, precipitated by ether, in water and precipitate the solution by neutral lead acetate.

Precipitate contains the lead salts of cholalic and glycocholic acids. Boil with alcohol, and evaporate the solution to dryness with sodium carbonate. Take up with alcohol, and precipitate the filtered liquid with ether; both acids give crystalline sodium salts. Sodium glycocholate forms six-sided prisms, with a single face having very oblique truncations. Agitate the liquid with dilute sulphuric acid and ether, and filter.	**Filtrate** contains taurocholic acid. Add a solution of basic lead acetate and ammonia, wash and convert the precipitate to the sodium salt. Boil the latter for six hours with hot saturated baryta-water. The taurocholic acid is hydrolysed, with the formation of *taurine* and *cholalic acid*. Filter the boiling liquid, and pass carbon dioxide through the filtrate. Filter again, and add hydrochloric acid to the filtrate.

Precipitate may contain *choloidic* and *glycocholic* acids. Choloidic or choleïc acid may be recognised by its characteristic resinous appearance, and by its precipitation as a resin on acidifying a solution of one of its salts.	**Filtrate** may contain *cholalic acid*, which may be recognized by its crystalline form, and by the characters of its barium salt.	**Precipitate** consists of *cholalic acid*.	**Filtrate.** Add sufficient sulphuric acid to precipitate any remaining barium. The excess of sulphuric acid is then removed by addition of lead hydroxide, and any lead which may have dissolved is precipitated by passing hydrogen sulphide. The solution is finally evaporated to dryness on the water-bath, and the residue taken up with alcohol. Any undissolved matter is *taurine*.

When it is merely desired to ascertain the amounts of glycocholic and taurocholic acids in a mixture of their sodium salts, this can be effected by oxidising a weighed quantity of the mixture with fuming nitric

acid, and converting the resultant sulphuric acid into barium sulphate, as described on page 413. 100 parts of $BaSO_4$ represents 225.3 parts of sodium taurocholate, and by deducting the amount thus found from that of the mixed sodium salts the weight of sodium glycocholate may be obtained.

Separation of Cholalic, Choleic and Deoxycholeic Acids.[1]

The crude acids obtained by the hydrolysis of ox-bile by sodium hydroxide is dissolved in dilute ammonia, and the solution which should not contain more than 5% of the ammonium salts, is warmed for a short time (10 to 20 min.) with animal charcoal. The acids are then precipitated from the solution of ammonium salts by dilute hydrochloric acid, and after drying *in vacuo* over soda lime and calcium chloride until the mass can be readily disintegrated to a fine powder, they are recrystallised from hot acetone. The mother liquors are concentrated and allowed to stand so long as crystals separate, each crop being rapidly washed on a filter pump with cold acetone to free it from adhering mother liquors. In this way 80% of the crude mass is obtained in the form of crystals of a light green tinge, which contain at least 90% of all the bile acids. The separation of the three acids in this mixture is effected by taking advantage of the fact that when the sodium salts in 1% solution are treated with 1/5 the volume of 20% magnesium chloride solution and warmed on a water-bath, and the mixture is allowed to cool, the great part of the cholalate remains in solution, whereas most of the choleic and deoxycholeic acids separate in the form of a crystalline magnesium salt. From this mixture of magnesium salts, the choleic acid can be separated in the form of a barium salt. The precipitation with barium chloride also carries down with it some deoxycholeate, partly in the form of its sodium salt. The precipitated barium choleate must, therefore, be thoroughly washed by grinding it up in a mortar after filtration and when still moist, with water. The filtrate from the barium salt, contains a mixture of cholalate and deoxycholeate, and the greater part of the latter can be separated by conversion again into sodium salt and treating the 1% mixture with magnesium chloride and warming as before. Nearly

SCHEME FOR SEPARATION OF THE UNCONJUGATED BILE ACIDS; CRUDE HYDROLYSIS PRODUCT RECRYSTALLISED FROM HOT ACETONE.

Crude crystalline mixture (80% of the total). *This is converted into the sodium salts which are treated in 1% solution with 1/5 of the volume of 20% MgCl₂ solution and warmed.*

Oily mother liquor containing fatty acids, pigments and small quantities of bile acids. *It is usually not worth while to prepare the bile acids from this, as it does not contain at the most more than 10% of the total.*

Precipitate of Mg salts containing about 1/5 of the cholalic acid and nearly all the choleic acid. *The free acids are regenerated from this, and the sodium salts prepared and treated in 2% solution with about 1/5 the volume of 20% BaCl₂ solution in the cold.*

Mother liquor A containing mostly cholalate with small quantities of deoxycholeate. *It is concentrated to 1/5 of its bulk.*

Precipitate. Mg deoxycholeate. *Mother liquor B united with mother liquor C.*

Precipitate is after thoroughly washing nearly pure barium choleate from which on treatment with acids is obtained **choleic acid.**

The mother liquor contains deoxycholeate and cholalate. *The free acids are regenerated by HCl and the sodium salts in 1% solution are treated with 1/5 the volume of 20% MgCl₂ solution (and warming).*

Combined mother liquors acidified with excess of hydrochloric acid yield **cholalic acid.**

The precipitate is mainly Mg **deoxycholeate.**

Mother liquor C *concentrated to 1/5 the bulk and after filtration is added to mother liquor B.*

pure magnesium deoxycholeate then separates. The mother liquors from the magnesium salt precipitation still contain appreciable quantities of the deoxycholeate, which separates on concentration of the solution to about 1/5 of the original bulk.

The general scheme is indicated in the accompanying table, and by this means the acids are separated into three main fractions containing respectively: cholalic, choleic and deoxycholeic acid. These fractions are not quite pure acid, but contain small quantities of other acids.

The cholalic acid fraction can be purified by recrystallisation from alcohol. The greater part separates out pure. From the mother liquors further quantities can be obtained by hydrolysing with excess of alkali hydroxide to destroy esters formed by the action of alcohol, and after evaporation of alcohol, precipitating the acids from aqueous solution of the sodium salts by acid. The crude product thus obtained still contains small quantities of choleic and deoxycholeic acids, which can be precipitated from a 2 % solution of the sodium salts of the mixed acids by a large excess of barium chloride. From the filtrate, the acid can be regenerated, which on recrystallisation from alcohol yields a further crop of pure cholalic acid.

The choleic acid, when present in small quantity can be recrystallised from acetone. In dealing with large quantities, it is best to recrystallise from alcohol. The alcoholic mother liquors are then hydrolysed with sodium hydroxide to destroy the ester formed, and the acid obtained after acidification with hydrochloric acid of the aqueous solution of the sodium salt is then recrystallised from acetone. This process causes great saving of solvent, and gives a good yield of pure acid. The deoxycholeic acid is obtained from the magnesium salt by grinding the latter with excess of dilute hydrochloric acid. It is recrystallised, after drying, from glacial acetic acid.

Pettenkofer's Reaction for Bile-acids.—The most delicate and characteristic reaction of cholalic acid is that known as Pettenkofer's test, but which could be more appropriately termed the furfural reaction. It depends on the vivid purple colouration produced on treating cholalic acid with strong sulphuric acid and furfural or any substance (such as sugar) capable of yielding furfural by reaction with the acid. The reaction is common to all the varieties of cholalic acid, and also to the conjugated forms in which they exist in bile from various sources. As the detection of bile, especially in urine, is often of considerable pathological importance, the reaction has a practical interest.

Pettenkofer's reaction is most simply observed by treating a drop of bile on a porcelain surface with a drop of a 10% solution of cane-sugar and adding a drop of strong sulphuric acid. A bright cherry-red colour will be produced, and, either at once or on gently warming the mixture, will rapidly change to a magnificent purple tint, ultimately becoming bluish. Too high a temperature must be carefully avoided, or the reaction will be obscured by the charring of the sugar, and excess of sugar should be avoided for the same reason. Hence it has been proposed to employ furfural instead of sugar in important cases. The red solution shows an absorption spectrum of two bands, one at F and the other between D and E near E. The sulphuric acid used should be as far as possible free from SO_2 and oxides of nitrogen.

In using furfural, 1 c.c. of the liquid to be tested, which may be either aqueous or alcoholic, is treated in a test-tube with 1 drop of a solution of furfural in 1,000 parts of water, 1 c.c. of concentrated sulphuric acid is then added, and the tube immersed in water so that the temperature does not exceed 50 to 60°.

The tendency to char which attends the use of cane-sugar may be avoided by employing dextrose in its place. If a little dextrose be dissolved in concentrated sulphuric acid, and a few drops of the freshly made reagent be allowed to fall in the centre of a small pool of urine on a white plate, the play of colours produced in the presence of bile-acids may be observed under very favourable conditions.

It has also been proposed to employ phosphoric acid in place of sulphuric acid, but the substitution is not desirable.

Unfortunately, Pettenkofer's reaction is not peculiar to the bile-acids. Udranszky has enumerated 76 organic substances which have somewhat similarly, but of these only α-naphthol gives the reaction as readily as the bile-acids. A useful confirmation of the reaction is afforded by the absorption-spectrum of the colouring matter. For this purpose the colour should be produced as already described, and the purple liquid diluted with glacial acetic acid or alcohol until the tint is of suitable depth for observation of the spectrum. The colouring matter from bile-acids exhibits four absorption-bands. Of these, the band slightly on the red side of the Fraunhofer line E, and another about F, are the best defined. Two others may be observed near D. On further dilution of the liquid, these two bands disappear entirely, and that between D and E becomes indistinct, but the most refrangible band still persists. The cherry-red colour produced by albumin,

when treated with sulphuric acid and sugar, shows one absorption-band, between E and F, and does not exhibit the dichroism characteristic of the colouring matter of the bile-acids.

The colouring matter formed in Pettenkofer's reaction is soluble in ether.

Pettenkofer's reaction is observed or actually falsified by proteins, fatty matters, and certain colouring and extractive matters, and hence it is important to remove these before employing the test. In applying the test to urine, purification is usually sufficiently effected by rendering the liquid distinctly but not strongly acid with acetic acid, boiling for a minute or two, and filtering from any mucus, albumin, etc., which may be precipitated.

In applying Pettenkofer's test to urine, it is often desirable to concentrate the liquid previously on the water-bath. A little cane-sugar or glucose is then dissolved in it, and a portion of the cold liquid placed in a test-tube. Strong sulphuric acid is then allowed to run down the·side of the tube so as to form a distinct layer below the urinous liquid, when the characteristic purple colouration will be developed at the junction of the two strata if any bile-acids be present. An alternative and very delicate mode of performing the test is to dip a slip of filter-paper in the sweetened urine, and allow it to dry spontaneously. When dry, a drop of concentrated sulphuric acid is applied to the paper by means of a glass rod, when if bile-acids be present, even to the extent of 0.03%, in less than half a minute a violet stain will be produced on the paper, which is best viewed by transmitted light.

In certain cases it is an advantage previously to isolate the bile-acids from the urine in an approximately pure condition before applying Pettenkofer's test. For this purpose the largest volume of urine available should be boiled, filtered, and treated with lead acetate and ammonia as long as a precipitate forms. The precipitate is filtered off, washed well, pressed and boiled with alcohol, which dissolves the lead salts of the bile-acids, leaving the urate, phosphate, etc., insoluble. The alcoholic liquid is filtered boiling hot, and evaporated on the water-bath with a few drops of a solution of sodium carbonate. From the residue the sodium salts of the bile-acids are dissolved by alcohol, and to the solution Pettenkofer's and other tests can be advantageously applied. To detect traces of bile-acids, the alcoholic solution of the soidum salts is concentrated to a few drops, and three or four drops

of dilute sulphuric acid (1:4) added, together with a minute quantity of cane-sugar or dextrose. The liquid is then evaporated at a gentle heat, when the characteristic violet colouration will be produced with as little as 0.0001 grm. of bile-acids.

To obtain the bile-acids pure so that Pettenkofer's test can be applied to them, the protein and fat must first be removed. The protein is removed by making the liquid first neutral and then adding a great excess of alcohol, so that the mixture contains at least 85% of water-free alcohol. Now filter, extract the precipitated protein with fresh alcohol, unite all filtrates, distil the alcohol, and evaporate to dryness. The residue is completely exhausted with strong alcohol, filtered, and the alcohol entirely evaporated from the filtrate. The residue is extracted with ether and dissolved in water, and filtered if necessary and the solution precipitated by basic lead acetate and ammonia. The washed precipitate is dissolved in boiling alcohol, filtered while warm, and a few drops of sodium hydroxide solution added. Then evaporate to dryness, extract the residue with absolute alcohol, filter, and add an excess of ether. The precipitate now formed may be used for Pettenkofer's test. It is not necessary to wait for crystallisation; but one must not consider the crystals which form in the liquid as being positively crystallised bile. It is also possible for needles of alkali acetate to be formed. In this connection it must be remarked that a confusion with phosphatids, which also give Pettenkofer's reaction is not excluded, and a further testing and separation is advisable. It must be remembered that while Pettenkofer's reaction is given by all the acids of bile, and by the acid products of their hydrolysis, no similar colouration is produced by taurine or by biliary pigments (see below). Medical men often fall into error on this point, and assume the absence of bile-pigments from urine because the sample gives a negative reaction with Pettenkofer's test. It is a fact that in jaundice the urine contains very little bile-acids, and frequently they are entirely absent, while on the other hand the bile-pigments are conspicuously present in the urine of jaundiced persons.

Bile-pigments.

Bile contains certain colouring matters, which are derived from hæmoglobin[1] and are not chemically related to the bile-acids.

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[1] The bile-pigments contain no iron, which, however, exists in the bile in the form of phosphate, and is deposited in comparatively large amount in the liver in cases of pernicious anæmia.

Bilirubin, $C_{16}H_{18}O_3N_2$, or $C_{32}H_{36}O_6N_4$, is the yellow pigment of the bile of man and herbivorous animals. It is troublesome to prepare pure, but is best obtained by extracting powdered human gall-stones with ether, which extracts cholesterol. The residue is boiled with water, and treated with dilute hydrochloric acid to decompose the calcium salt, $Ca(C_{16}H_{17}O_3N_2)_2$, which is the form in which bilirubin occurs in gall-stones. The mass is washed, dried, and extracted with chloroform, the chloroform distilled off, and the residue treated with absolute alcohol. It is then again dissolved in chloroform, and precipitated by absolute alcohol.

Bilirubin is an orange powder, insoluble in water, alcohol, or ether, but soluble with some difficulty in benzene and chloroform. It dissolves in alkalies with orange colour, and is precipitated unchanged if hydrochloric acid be at once added, and may be extracted by agitation with chloroform; but if the alkaline liquid be exposed to the air it gradually absorbs oxygen, and then yields a green precipitate of biliverdin, $C_{16}H_{18}O_4N_2$, when acidified. It can be obtained as crystals from its solution in hot dimethylaniline. If the oxidation be carried further, as by adding yellow nitric acid to an alkaline solution of bilirubin mixed with an equal measure of alcohol, a blue pigment, bilicyanin, is formed; next a violet, which is perhaps a mixture of the red and blue; then a red colouring matter; and lastly a yellow pigment, called by Maly *choletelin*, and said to have the composition $C_{16}H_{18}O_6N_2$.

The foregoing colour-reactions may be conveniently observed by spreading a drop of bile in a thin film on a porcelain plate, and placing a drop of yellow nitric acid in the centre, when a series of rings will be produced, coloured successively green, blue, violet, red, and yellow. By placing the platinum terminals of a battery of four Grove's cells in some bile, the succession of colour-reactions due to the oxidation of bilirubin will be produced round the anode, and can be observed to great advantage. On reversing the current the colour-changes occur in the opposite order.

By the reduction of bilirubin in alkaline solution by means of sodium amalgam, Maly obtained hydrobilirubin, $C_{32}H_{40}O_7N_4$, or $C_{32}H_{36}O_5N_4 + 2H_2O$, said by some observers to be identical with urobilin, the colouring matter of normal urine. Hydrobilirubin is also identical with, or closely related to, stercobilin, the colouring matter of fæces in a state of health, but is absent during an attack of jaundice, when the fæces are slate-coloured

Biliverdin, $C_{16}H_{18}O_4N_2$, exists in human bile, but is especially characteristic of the bile of herbivorous animals. It is produced with great facility by the oxidation of bilirubin, from which it differs in colour (dark green), its insolubility in chloroform, and its ready solubility in alcohol and glacial acetic acid. It is also soluble in benzene and in carbon disulphide, but is only slightly soluble in ether. If yellow nitric acid be added to an alcoholic solution of biliverdin a bluish-violet colouration is produced, changing to red and finally to yellow with excess of acid.

Detections of Bile-pigments in Urine.—The colouring-matters of bile are not present in normal urine, but in certain diseases (jaundice, etc.) they exist in very appreciable amount. Such urine exhibits a yellowish-green, green, greenish-brown, or almost black colour; bilirubin predominates in bilious urine of a saffron yellow colour; while biliverdin and other oxidation products are present in greenish urine. Bilious urine gives a yellow froth on agitation, and stains linen and filter-paper yellow.[1]

A variety of tests have been proposed for the detection of bile-pigments in urine, but the following are the most delicate, and answer every purpose:

Gmelin's test consists in treating the urine with strong nitric acid and observing the change of colour produced. The reaction is best observed by allowing some of the urine to run gently on to the surface of some yellow nitric acid contained in a test-tube. If bile-pigments be present, a green ring will become apparent at the point of contact, while below this will appear violet, red, and yellow zones, in the order named. The green colour alone is characteristic of bilious urine, since indigogens give rise to blue and red colourations. The urine of patients who have taken potassium iodide also gives a red zone with nitric acid. Various modifications of Gmelin's reaction have been proposed.

Hammarsten's Test.—A mixture of 1 volume nitric acid and 19 volumes hydrochloric acid (both acids about 25%) is made and when allowed to stand until yellow in colour, 1 volume of this acid mixture is added to 4 volumes alcohol. If a solution containing bile pigments is added to a few c.c. of this colourless solution a series of colours corresponding to the colours obtained in Gmelin's test is obtained.

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[1] If the dyed filter-paper be treated with a drop of nitric acid, the margin of the spot will become violet or deep blue, while the centre gradually changes to emerald green.

In the presence of blood or other pigments it is best to place about 10 c.c. of the acid or nearly neutral (not alkaline) fluid in a tube and adding borium chloride solution and centrifuging for a few minutes. The supernatant fluid is decanted and the sediment treated with 1 c.c. of the above reagent and centrifuged again. A beautiful green solution is obtained and on adding more of the reagent it becomes blue, violet, red and reddish-yellow. A green colour can be obtained in a urine containing 1 part of pigment in 500,000 to 1,000,000 parts urine. Calcium chloride is used to a better advantage in the presence of large quantities of other pigments.

Huppert's Test.—If a solution of alkali bilirubin is treated with milk or lime or with calcium chloride and ammonia, a precipitate is produced consisting of calcium bilirubin. If this moist precipitate, which has been washed with water, is placed in a test-tube and the tube half filled with alcohol which has been acidified with hydrochloric acid, and heated to boiling for some time, the liquid becomes emerald-green or bluish-green in colour. On applying this test for bile pigments in urine it is treated with calcium hydroxide or first with calcium chloride and then a solution of sodium or ammonium carbonate and the precipitate collected on a filter and treated as above described.

Rosin's test for bile-pigments consists in allowing very dilute iodine solution or bromine-water to flow on the surface of the urine from a pipette. A glass-green ring is produced at the junction of the two strata.

Another reliable test for bile-pigments in urine is to treat 30 c.c. (or 1 oz.) of the sample with about one-third of its bulk of a 20% solution of zinc acetate, after previously neutralising most of the free acid by sodium carbonate. The voluminous precipitate is filtered off, washed, and treated with a little ammonia. In presence of bile-pigments the ammoniacal liquid is usually fluorescent, and either at once or on standing shows the absorption-spectrum of bilicyanin, characterised by bands on each side of the D line, and a third between B and F.

It must be remembered that some urines which contain when fresh, only a small amount of bile-pigment, will after being exposed to the air for several days, show no bilirubin whatever, urobilin having taken its place.[1] The source of urobilin in the fæces is also doubtless

[1] Fresh urine containing but little urobilin often becomes darker on exposure to the air, a change probably due to the formation of urobilin from a substance called by MacMunn urobilinogen.

the bile-pigment, unaltered bile-pigment never occurring in normal fæces.

Urinary urobilin exhibits a green fluorescence when the urine is rendered ammoniacal, and a few drops of zinc chloride are added. It shows a well-marked absorption band between the Fraunhofer lines b and F. The coloured products formed from bilirubin by oxidation, with the exception of the final yellow product choletelin, all exhibit a similar fluorescence with ammonia and zinc chloride, and show an absorption-band near F; but less sharply defined than in the spectrum of urobilin which, however, they closely resemble.

From some urines pigments can be separated which possess all the characters of the red and brown oxidation products of bilirubin, while others yield a substance identical with choletelin. Hence the colouring matters of normal urine, which may be termed physiological urobilins, are oxidation-products of bilirubin, while pathological urobilins are reduction-products of the same substance. Pathological urobilins are produced in some cases from bilirubin, but can be formed from blood-pigment directly after extravasation of blood.

LACTIC ACID.

By W. A. DAVIS.

Two chemically isomeric forms of hydroxypropionic acid exist having the structures

$$\begin{array}{ccc} CH_3 & & CH_2.OH \\ | & & | \\ CH.OH & and & CH_2 \\ | & & | \\ CO_2H & & CO_2H \end{array}$$

α-Hydroxypropionic acid.   β-Hydroxypropionic acid.
Lactic acid.   Hydracrylic acid.
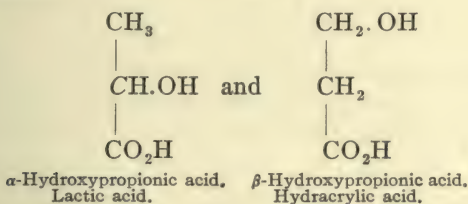
α-Hydroxypropionic acid, moreover, by virtue of its containing an asymmetric carbon atom (indicated in italics) exists in three optically different forms, viz., dextro-lactic acid (sarcolactic acid), lævo-lactic acid, and inactive or ordinary (fermentation) lactic acid. Of these substances, the last is by far the most important.

Inactive Lactic Acid, $C_3H_6O_3$ or $CH_3.CHOH.CO_2H$.

Lactic acid was first obtained by Scheele, in 1780, from sour milk. It exists ready-formed in both the animal and the vegetable kingdoms. Lactic acid is formed by the lactic acid fermentation of carbohydrates such as sugar, gum, starch, mannitol, and particularly of milk-sugar, in the presence of casein or other proteins. Hence lactic acid is contained in sour milk (but not in fresh milk), in sourkrout, pickles, distillery-wash,[1] sour beer, etc. The acid contained in sour tan-liquors and the acid runnings of starch-makers, etc., called by Braconnot nanceic acid, and the so-called thebolactic acid contained in opium are also ordinary lactic acid. Lactic acid is present in molasses (Schöne and Tollens, *Zeit. Ver. deutsch. Zuck. Ind.*, 1900, 980; Weisberg, *Bull. Assoc. Chim. de Suc.*, 1901, **18,** 545).

Lactic acid is obtainable by various synthetical processes, including:

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[1] Spent distillery wash, technically called pot-ale or burnt ale, contains about 3 % of solid matters, of which about 1 % consists of lactic and other acids, 0.7 of peptones and other nitrogenous matters, and 0.7 % mineral matters, in which phosphates predominate.

The action of nitrous acid on alanine; the oxidation of α-propylene-glycol by nitric acid; the action of alkalies on α-chloro- or bromo-propionic acid; the reaction of aldehyde with hydrocyanic acid, and treatment of the resulting hydroxycyanide with hydrogen chloride; the cautious oxidation of glycol with spongy platinum or dilute nitric acid; the reduction of pyruvic acid, $CH_3.CO.CO_2H$, etc.

It is also formed in considerable quantity by the action of alkalies on certain carbohydrates under suitable conditions, more particularly in absence of air or oxidising substances (see Kiliani, *Ber.*, 1882, **15,** 699). Thus invert sugar yields with alkalies 50 to 60% of lactic acid (together with 0.5 to 2% of formic acid and 40 to 50% of polybasic acids). Galactose yields only 20% of lactic acid (Meisenheimer, *Ber.*, 1908, **41,** 1010).

Lactic acid is a regular constituent of wine and until recently it was thought that it was an intermediate product in the alcoholic fermentation of sugar. But the fact that the acid is not fermentable by yeast (Slator, *Trans.*, 1906, **89,** 141; 1908, **93,** 231; Buchner and Meisenheimer, *Ber.*, 1910, **43,** 1773), makes the hypothesis improbable. Its presence in wine is apparently to be attributed to the fermentation of malic acid by the *Micrococcus malolacticus* (Seifert, *Chem. Centr.*, 1907, ii, 346; Rosenstiehl, *Compt. rend.*, 1908, **147,** 150).

Lactic acid is commercially prepared by the lactic fermentation of sugar (for details see Claflin, *J. Soc. Chem. Ind.*, 1897, **16,** 516; Shafer, *Chem. Zeit.*, 1907, **6,** 177 and 189; McLauchlan, Int. Congress Appl. Chem., 1909, Section IV a, **1,** 141).

The formation of lactic acid by the fermentation of glucose may be represented by the equation $C_6H_{12}O_6 = 2C_3H_6O_3$, but the action is much less simple than is thus indicated. Numerous organisms are capable of converting sugar into lactic acid, but that known as the lactic ferment (*Bacillus acidi lactici*), especially the Bulgarian variety, has by far the most energetic action. It consists of short thick cells generally united in pairs, and is most active between 35° and 45°. Access of air is necessary, and nitrogenous food is required. Excess of acid arrests the fermentation, but the action recommences if the liquid be neutralised. The action is not affected by 2% of alcohol, but 4% diminishes it, and 6% arrests it completely.

To prepare lactic acid in the laboratory the following method is given by McLauchlan. It is similar in principle to the method followed on the large scale: 400 grm. of commercial glucose (70% grape sugar) is

dissolved in 1,200 c.c. of hot water, 170 grm. of powdered calcium carbonate added and 20 grm. of malt which has previously been boiled a few minutes with 100 c.c. of water. The mixture is then kept at a gentle boil during 30 minutes in order to sterilise the raw material, cooled to 60 to 70° and after stirring well, is poured into a narrow-mouthed bottle of a capacity of 2 to 2.5 litres. Any calcium carbonate is rinsed out with water until the volume of liquid amounts to 1,800 c.c. The bottle is then stoppered with a wad of cotton wool and placed in an incubator maintained at a constant temperature of 45°. The fermentation is started by means of a pure culture of the lactic ferment, which may be prepared as follows: Sweet milk is sterilised in a number of test-tubes, which are then closed with a plug of cotton-wool and placed in the incubator. Tube No. 1 is inoculated with a drop of sour milk and one day later tube No. 2 is inoculated from tube No. 1; then on the next day tube No. 3 is inoculated from No. 2 and the process repeated until a pure culture is obtained in the seventh tube.

A few grams of the pure culture are added to the sugar mixture and after a few hours the progress of the fermentation becomes visible by the evolution of carbon dioxide when the bottle is shaken. The bottle should be shaken every 6 hours or so and after a week a sample should be tested daily, by means of Fehling's solution, for the percentage of sugar remaining in solution. When this falls below 0.4% the fermentation is finished. The calcium lactate is poured into a large beaker and sulphuric acid equivalent to the calcium present added. After stirring well, a test-tube full is filtered and tested with a drop of sulphuric acid to ascertain whether sufficient acid has been added to precipitate all the calcium as calcium sulphate. When this point has been reached the calcium sulphate is removed by filtration on the pump and the solution concentrated, preferably *in vacuo*, when more calcium sulphate separates and is removed by filtration through charcoal which decolourises the liquid. The final filtrate should amount to 1 litre of 25% lactic acid.

A purer lactic acid can be obtained by cooling the solution obtained by fermentation so as to obtain crystals of calcium lactate which is then recrystallised and the product decomposed by sulphuric acid.

Commercial Lactic Acid.—In America lactic acid is sold generally in the form of solution with an acidity representing 25% of lactic

acid.   A solution with a concentration of 50% is also widely used, more particularly in England and Germany.   The lactic acid of the British and United States Pharmacopœia has a sp. gr. of about 1.21 (see below) and is supposed to contain 75% of lactic acid.   It also contains about 8% of lactic anhydride.

Pure anhydrous lactic acid, which is obtained by repeated fractionation of the concentrated liquor in a vacuum of 0.5 to 1 mm. forms a crystalline but very hygroscopic mass which melts at 18° and boils at 82 to 85° under the pressure given and 119 to 120° under 12 mm.

Lactic anhydride and lactide are formed when lactic acid is evaporated at the ordinary temperature in dry air.   The following table shows the percentage composition of products thus obtained by Wislicenus:

	Water	Lactic acid, $C_3H_6O_3$	Lactic anhydride, $C_6H_{10}O_5$	Lactide $C_6H_8O_4$
A. Freshly prepared as above; syrup.........	15.64	58.80	25.56	....
B. After drying 4 months over sulphuric acid.	4.07	22.43	73.50	....
C. After 13 months; thick syrup insoluble in water.	....	....	97.85	2.06
D. After 16 months; treacly syrup...........	....	....	71.41	28.69
E. After 18 months; thick gummy mass......	....	....	60.77	39.50

Lactic acid is not volatile under ordinary pressures without decomposition.   At 130° it begins to decompose, and at about 145° sparingly soluble lactic anhydride is formed, which at a higher temperature forms lactide and other products.

Lactic anhydride and lactide are nearly insoluble in water, but are converted by prolonged boiling with water, and readily by solutions of alkali hydroxides into lactic acid.   The lactide obtained by heating paralactic acid (dextrorotatory) yields ordinary inactive lactic acid when treated in this manner.

Lactic acid is miscible in all proportions with water, alcohol, glycerol, and ether.   It is but slightly soluble in chloroform, and is insoluble in carbon disulphide and petroleum spirit.   Glyceric acid, which resembles lactic acid, is insoluble in ether.

Lactic acid dissolves freshly precipitated calcium phosphate, and is frequently used for that purpose.

Concentrated sulphuric acid mixes with pure lactic acid without blackening it.   On heating, a brown colour is developed, and much carbon monoxide evolved, a humus-like substance being ultimately

left. When heated with dilute sulphuric acid a mixture of acetaldehyde and formic acid is obtained.

On distillation with a large excess of quicklime, lactic acid is converted into carbon dioxide and alcohol.

Lactic acid does not reduce Fehling's solution, but rapidly decolourises potassium permanganate, both in acid and in alkaline solutions with production of an odour of aldehyde. Silver lactate is imperfectly reduced on boiling, with production of a blue liquid and a brownish deposit.

Lactic acid may be separated from organic acids forming insoluble lead salts by precipitating the solution (previously neutralised if necessary) with neutral lead acetate, either with or without an addition of alcohol. Lead lactate remains in solution and may be decomposed by hydrogen sulphide, when free lactic acid is obtained.

Many admixtures may be separated from lactic acid by saturating the free acid by barium carbonate. When the aqueous solution is evaporated and the residue treated with alcohol, many of the acids whose barium salts are soluble in water remain behind, whereas barium lactate dissolves in alcohol. Free lactic acid may be obtained by cautiously precipitating the solution of barium lactate with dilute sulphuric acid, and filtering.

When purified from all substances except those soluble in alcohol, the aqueous liquid containing free lactic acid may be saturated with oxide of zinc, evaporated to dryness, and the residue digested with alcohol. Zinc lactate insoluble in alcohol, remains, while the other matters dissolve. After drying at 120°, the residue may be weighed, when its weight, multiplied by 0.7400, gives that of the lactic acid. Zinc sarcolactate dissolves readily in alcohol, so the above process is useless for the estimation of sarcolactic acid. With inactive lactic acid it yields fairly approximate results, with careful manipulation and under favourable circumstances.

According to R. Palm (*Zeit. anal. Chem.*, 16, **33**), when treated with lead acetate and alcoholic ammonia, lactic acid is completely thrown down as a heavy granular precipitate of the formula $3PbO, 2C_3H_6O_3$. To examine an animal or vegetable organ for free lactic acid, Palm extracts it with ether (previously acidifying with sulphuric acid if a lactate is under treatment), evaporates the ethereal solution to a syrup, and treats the residue with water. The filtered aqueous solution is mixed with lead acetate, and any precipitate produced

is filtered off. On adding more lead acetate to the filtrate, followed by alcoholic ammonia, the lactic acid is thrown down free from foreign substances. The precipitate may be washed with alcohol, in which it is quite insoluble, and the contained lactic acid estimated from the loss on ignition. Minute traces of lactic acid may be thrown down with greater certainty by shakng the filtrate from the first lead precipitate with an excess of freshly precipitated lead hydroxide. In either case the precipitate is stated to yield pure lactic acid when decomposed by hydrogen sulphide and extracted with ether.

Calcium lactate is rapidly fermented by certain bacteria, giving rise to volatile fatty acids; in some cases butyric acid is the principle product, in others propionic and *n*-butyric acid predominate.

Qualitative Tests.

Windisch (*Chem. Centr.*, 1887, 826) proposed to detect small quantities of lactic acid by treating the substance with chromic acid, whereby formic acid and aldehyde are produced. The solution to be tested is diluted to about 100 c.c., 5 c.c. of concentrated sulphuric acid and a little potassium dichromate added, and the liquid distilled. The vapours are received in warm Nessler's solution, with which, in presence of aldehyde, lead salts give a yellowish-red precipitate, or with smaller quantities a yellowish opalescence. Formic, acetic, propionic, butyric, valeric, succinic, malic, citric, and tartaric acids are said not to give the reaction, but alcohol, ammonia, and sugar must be absent. Small quantities of lactic acid are liable to escape detection by this method, owing to the oxidation of aldehyde to acetic acid by excess of chromic acid. To examine roots for lactic acid, they are first exhausted with ether, which is said to extract all substances of an acid nature.

A sensitive test for lactic acid is given by Denigès (*Bull. Soc. Chim.*, 1909, [iv], **5,** 647) which is based on the formation of acetaldehyde on heating lactic acid with sulphuric acid. 0.2 c.c. of a solution of lactic acid (up to 2% strength) is heated with 2 c.c. of sulphuric acid (sp. gr. 1.84) at 100°, during 2 minutes. To the cold mixture a drop of an alcoholic solution of guaiacol or codeine is added; with the former a rose-red tint is produced and with the latter an orange-red colouration. This test serves to distinguish lactic acid from glycollic acid, which gives rise under the conditions named to formaldehyde, and hence

produces different colourations from those shown by lactic acid.

A test due to Vournasos (*Zeit. angew. Chem.*, 1902, **15**, 172) and modified by Croner and Cronheim (*Berl. klin. Wochenschr.*, 1905, **42**, 1080) for lactic acid is based on its conversion into iodoform and of the latter into phenyl isonitrile, which can be recognised when present in very small quantity by its smell. 2 grm. of potassium iodide are dissolved in water, 1 grm. of sublimed iodine added and the solution filtered through glass wool or asbestos, and made up to 50 c.c. A few c.c. of the liquid to be tested are made alkaline with 10% potassium hydroxide solution after which the liquid is boiled for a few minutes and then mixed with the aniline-iodine solution. The presence of lactic acid is shown by the well-known isonitrile odour being detected. Care must of course be taken that no other substances (*e.g.*, alcohol, acetone, etc.), which are capable of giving rise to iodoform under the conditions named, are present. If such substances are present the test is valueless.

According to Thoms (*Zeit. physiol. Chem.*, 1907, **50**, 540) the following test is preferable as it is not shown by butyric acid, acetic acid, acetone, alcohol or hydrochloric acid. The gastric extract is heated with a few drops of 30% chromic acid for 10 minutes on the water-bath, when a reddish-brown colour is given by traces of lactic acid.

Fletcher and Gowland Hopkins (*J. Physiol.*, 1907, **35**, 247) recommend the following test: 5 c.c. of sulphuric acid, a drop of a saturated solution of calcium sulphate and a few drops of the suspected solution are heated in a test-tube placed in a water-bath for 2 minutes. The tube is cooled and 2 to 3 drops of a dilute solution of thiophen (10 to 50 drops in 100 c.c. of alcohol) added. On again heating in the water-bath, the fluid becomes light cherry-red in colour if lactic acid is present. This is due to an aldehyde reaction with thiophen.

For the detection of free lactic acid in gastric juice, Uffelmann (*Zeit. klin. Med.*, **7**, 392) prepared a reagent by mixing 10 c.c. of a 4% solution of phenol with 20 c.c. of water, and adding 1 drop of solution of ferric chloride, British Pharmacopœia. This forms a clear liquid of an amethyst colour, which is turned yellow by a solution of lactic acid containing only 1 part in 10,000. Hydrochloric acid in small quantities has but little effect, and when present in large amount simply decolourises the reagent. The test is preferably applied to the ethereal extract obtained as described on page 451, as in that case the traces of thiocyanates normally present in the stomach do not interfere.

This test is not, however, characteristic of lactic acid, but is responded to by tartaric, citric, malic and oxalic acids (Kühl, *Milchw. Zentr.*, 1910, **6,** 61). It gives negative results with benzoic and lævulic acids. If used for the detection of this group of acids it may be improved by substituting a 1/400% solution of salicylic acid for the phenol. Benzoic acid may also be used as follows: 5 c.c. of a 0.002% solution are mixed with a drop of ferric chloride (1:10) when a white turbidity with a green reflex is formed. On adding a few drops of a weak solution (0.001%) of lactic, tartaric, citric, malic or lævulic acid, the liquid becomes clear and turns a dark greenish colour. The reaction is also given by ammonium oxalate.

Separation of Lactic Acid from Other Organic Acids.

According to Schoorl (*Zeit. angew. Chem.*, 1900, **15,** 367) succinic, malic and lactic acids are separated from oxalic, tartaric and citric acids by adding excess of milk of lime, boiling and filtering. After evaporating to dryness, 70% alcohol extracts calcium lactate with traces of benzoate and salicylate if present. The residue contains malate and succinate. Lactic acid is oxidised to acetaldehyde by potassium permanganate and dilute sulphuric acid (malic acid also gives acetaldehyde). By dissolving calcium lactate in dilute acetic acid and adding cobalt or zinc acetate, the corresponding lactate is obtained by slow evaporation in the air and recognised by its crystalline form. Formic, acetic, benzoic and salicylic acids are volatile with steam and are thus easily separated from the less volatile lactic acid; it must be remembered, however, that lactic acid is slightly volatile with steam (Müller, *Bull. Soc. Chim.*, 1896, **15,** 1206. Partheil, *Zeit. Nahr. Genussm.*, 1902, **5,** 1053. Utz., *Chem. Zeit.*, 1905, **29,** 363).

According to Schneider (*Zeit. anal. Chem.*, 1899, **38,** 775) lactic, butyric and valeric acids are separated by distilling with superheated steam, when butyric and valeric acids pass over leaving lactic acid in the residue. To identify the lactic acid the contents of the retort are evaporated with zinc oxide and filtered while hot. Zinc lactate crystallises on cooling. A few centigrm. of the zinc lactate are mixed with phosphoric acid and extracted with ether. The ether is evaporated and a little cobalt acetate and lead acetate added; a precipitate is obtained of cobalt lead lactate in the form of colourless, hexagonal plates.

Estimation of Lactic Acid.

Numerous methods have been described for this purpose, but none seems entirely free from objection. In most cases the difficulty arises from the presence of other substances which are liable to be returned as lactic acid owing to their behaving in the same manner as this substance in the conditions of the experiment. The method of Chapman and Smith, for example, described in the last edition of this work, gives unreliable results because of the presence of other easily oxidisable substances.

Buchner and Meisenheimer's Method.—According to Buchner and Meisenheimer (*Ber.*, 1908, **41,** 1416 and 1910, **43,** 1784) the most accurate method of separating and estimating lactic acid when present with other substances is as follows:

10 c.c. of the solution (or an amount of material containing about 1 grm. of lactic acid) is mixed with 10 c.c. of dilute sulphuric acid and 10 c.c. of saturated sodium sulphate solution and extracted continuously during 72 hours with ether in a von der Heide extraction apparatus (*Ber. K. Lehranstalt f. Weinbau in Giesenheim*, 1906, 253). The ethereal extract is dried during 18 hours with anhydrous sodium sulphate, the solution filtered from the sodium sulphate and the latter thoroughly washed with ether. The ethereal filtrate is evaporated to a thick syrup (to remove volatile acid), which is taken up with water and boiled with zinc carbonate. After filtering and thoroughly washing the residual zinc carbonate with water, the solution is evaporated to 5 c.c., 15 c.c. of alcohol added, and the solution left to crystallise overnight. The crystals are then collected on a Gooch crucible, washed with 75% alcohol, and dried at 105°. The result is calculated from the relation $Zn(C_3H_5O_3)_2 = 2C_3H_6O_3$, and is apparently 5 to 10% low. Thus 0.985 grm. of pure lactic acid taken gave a result 0.934.

If non-volatile acids be present which form zinc salts that are sparingly soluble in dilute alcohol the method is useless.

If in the above method formic acid be present, there is danger of zinc formate crystallising from the alcoholic solution with the zinc lactate, unless the formic acid has been completely expelled during the evaporation of the ethereal solution to a syrupy consistence. On the other hand, if the heating of this syrup be continued too long there is danger of loss of lactic acid by volatilisation; this is probably the

reason of the low results recorded. If volatile acids or alcohols be removed by steam distillation prior to the ether extraction there is also danger of loss of lactic acid.

The above method has the advantage that from the proportion of water of crystallisation and of zinc oxide in the zinc lactate obtained it can be ascertained whether the lactic acid is the optically inactive or active form (inactive = $3H_2O$; active = $2H_2O$). The polarimeter can be used to distinguish between the d-and-l forms.

Kunz' Method.—This method (*Zeit. Nahr.-Genussm.*, 1901, 4, 673) was devised for estimating lactic acid in wine. For this purpose 20 c.c. of wine are mixed with a slight excess of powdered barium hydroxide and evaporated to about two-thirds of the original volume. When cold the whole is rinsed into a 200 c.c. flask, made up to the mark, and filtered; 150 c.c. of the filtrate are evaporated to a thin syrup after neutralising the excess of baryta with carbon dioxide, and, when cold, excess of dilute sulphuric acid is added and the whole introduced into a Schacherl extraction apparatus and extracted with ether during 18 hours [Partheil recommends the use of his own special form of extraction apparatus (*Zeit. angew. Chem.*, 1901, 121, 1020; *Ber.*, 1901, 34, 3611) for this purpose]. 30 c.c. of water are added, the ether driven off by warming, and the aqueous solution distilled in a current of steam until all the volatile acids have passed over. The residue after adding a few drops of phenolphthaleïn is mixed with a slight excess of barium hydroxide. If after 15 minutes' warming on the water-bath the alkaline reaction is still persistent, a current of carbon dioxide is passed and the filtrate concentrated to 10 c.c. It is then introduced into a 150 c.c. flask, using 40 c.c. of water for rinsing, and the liquid made up to the mark with 95% alcohol. 100 c.c. of the filtrate are evaporated on the water-bath and the residue rinsed into a beaker, acidified with hydrochloric acid and precipitated with sodium sulphate. From the weight of the barium sulphate obtained the corresponding weight of the lactic acid in 100 c.c. of wine is easily calculated.

In 23 samples of wine examined in this way, the lactic acid was found to vary between the limits 0.1185 and 0.4360 grm. per 100 c.c.

A somewhat different method for the same purpose was given by Möslinger (*Zeit. Nahr.-Genussm.*, 1901, 4, 1120), but according to Trummer (*Zeitsch. landw. Vers.-wesen. Oester.*, 1908, 11, 492) that of Kunz gives the most accurate results. It must be noted that the

methods of both Kunz and Möslinger assume that lactic acid is not volatile with steam, which apparently is not strictly the case (see Utz, *loc. cit.*). This is a possible source of error in both methods.

Jerusalem's Method.—In this method (*Biochem. Zeitsch.*, 1908, **12,** 361 and 379) the lactic acid is estimated by oxidising it to aldehyde according to the equation $2CH_3.CH(OH).CO_2H + O_2 = 2CH_3.CHO + 2CO_2 + 2H_2O$. The aldehyde is estimated by treating with an excess of iodine in alkaline solution and subsequently ascertaining the amount of iodine not absorbed to form iodoform. To get trustworthy results (*vide infra*) the oxidation of the aqueous solution of the acid must be carried out by warming the liquid containing the lactic acid with sulphuric acid and adding potassium permanganate to the boiling solution. The aldehyde as it is formed must be removed as quickly as possible and this is effected by means of a current of air. The apparatus for the absorption of the aldehyde must be very efficient and in the original a specially devised form is described. In estimating lactic acid in animal tissues and liquids by this method, as, for example, in blood, milk and urine, the lactic acid must be first extracted with ether. Quantitative extraction is only possible when the liquid is so concentrated as to be in a pasty consistency. Details are given in the original of a special apparatus suitable for the extraction.

It is clear that this method is liable to a number of sources of error, and is useless in presence of substances which yield acetaldehyde on oxidation. It is also necessary to ensure that none of the aldehyde is oxidised to acetic acid. According to von Fürth and Charnass (*Biochem. Zeitschr.*, 1910, **26,** 199) the method is untrustworthy except under very special conditions, such as the preservation of great dilution and low temperature. By maintaining certain standard conditions, which are defined in the paper referred to, a definite percentage of the theoretical yield of acetaldehyde can be obtained and the method can thus be made quantitative, but for this purpose the aldehyde should be estimated by Ripper's potassium hydrogen sulphite method (*Monatsh.*, 1900 **21,** 1079).

Partheil's Method.—This method (*Zeit. Nahr.-Genussm.*, 1902, **5,** 1053; see also *Ber. Deutsch. Pharm. Ges.*, 1903, **13,** 304) was devised to separate lactic acid from other volatile acids. It serves to estimate lactic acid in the mixture of volatile acids obtained from wine by distillation with steam. For this purpose the mixture of acids is heated for 30 minutes with an excess of baryta water, evaporated to

a small bulk and then transferred to a small distilling flask. The liquid is evaporated to complete dryness in a vacuum. After cooling, a tap funnel is fitted to the neck of the flask and the side tube connected with a nitrometer filled with potassium hydroxide solution. A few c.c. of concentrated sulphuric acid are introduced into the flask and the levels adjusted in the nitrometer. The contents of the flask are then heated carefully and the carbon monoxide formed, according to the equation $CH_3.CHOH.CO_2H = CH_3.CHO + H_2O + CO$, is measured. The number of c.c. of carbon monoxide reduced to 0° and 760 mm., multiplied by 0.00402 gives the weight of lactic acid in grm. The quantity of lactic acid present in the volatile acids of wine is about 0.04%. It is of course essential for this method to be trustworthy, that other substances, for example citric acid, which give rise to carbon monoxide, should be absent.

Paris (*Chem. Zent.*, 1908, [i], 773) approves of this method as giving good results, but Buchner and Meisenheimer (*loc. cit.*), state that it always gives low results. In the most favourable case they found it to be 7% low. It must be remembered, too, that Bistrzycki and Sie- meradzki (*Ber.*, 1906, **39,** 51) have stated that when lactic acid is heated with concentrated sulphuric acid it liberates only 80 to 85% of the theoretical quantity of carbon monoxide. On the other hand, it must be pointed out that while Buchner and Meisenheimer in condemning Partheil's method in their first communication (*Ber.*, 1908, **41,** 1416) state that their own method gives nearly theoretical results, in their 1910 communication they give examples which show a deficiency of 5 to 10%. From this it would appear that there is little to choose between the two methods on the score of accuracy, while in most cases the method of Partheil is certainly the more rapid. Further work on the relative value of the two methods is desirable.

Palm's Method.—The method of Palm described on page 433 has been used as a means of estimating lactic acid and is especially applicable in a few cases. But according to Ulzer and Seidel (*Monatsh.*, 1897, **18**, 138), it does not give concordant results.

Commercial Lactic Acid.

Lactic acid is largely used in dyeing and mordanting as a substitute for tartaric and citric acids, over which it possesses certain advantages. It also finds use in spirit distilleries, in tanning and for other purposes.

The following tests are made for the commercial valuation of the commercial acid (Mc Lauchlan, *7th Intl. Congress Appl. Chem.*, 1909, 148, and *J. Amer. Leather Chem. Assn.*, 1907, **15**).

(*a*) *Free Acid.*—20 grm. of commercial lactic acid are diluted to 250 c.c. and 25 c.c. of the dilute solution titrated direct, as rapidly as possible (cold) with $N/5$ sodium or potassium hydroxide, using phenolphthaleïn as indicator. 1 c.c. $N/5$ KOH = 0.0181 grm. lactic acid. The end-point is taken when a pink colour appears which remains on stirring.

(*b*) *Anhydride.*—25 c.c. of the same dilute solution are boiled with a known excess of $N/5$ sodium hydroxide for 10 minutes and then after adding sufficient $N/5$ acid to more than neutralise the sodium hydroxide, the excess of acid is titrated back with the alkali. The difference between the first and second estimations gives the anhydride present. This is generally from 5 to 10% of the total acidity, but may be higher.

(*c*) *Volatile Acids.*—These are estimated by evaporating 25 c.c. of the dilute solution to half its volume diluting to 20 c.c., repeating the operation two or three times and finally titrating the residual material, with $N/5$ sodium hydroxide using phenolphthaleïn. The difference between results *a* and *c* gives the volatile acids. This method is not very accurate owing to the volatility of lactic acid, but it answers trade purposes.

(*d*) *Ash* and *sulphuric acid* can be estimated in the ordinary way. If free sulphuric acid or volatile acids are present the value for the acidity in *a* does not of course represent lactic acid, and allowance has to be made accordingly.

According to Besson (*Collegium*, 1910, 73; *Chem. Zeit.*, 1911, **35**, 26) the process of estimating lactic anhydride by boiling is not reliable as the results obtained vary greatly, according to the time of boiling, the excess of alkali used, etc. The following method is recommended instead, and appears to give reliable results: To an aliquot proportion of the dilute solution of lactic acid phenolphthaleïn as added and the liquid titrated with $N/1$ ($N/2$ or $N/4$) alkali hydroxide until the red tint appears; then, according to the concentration, 1 to 3 c.c. of normal alkali (or the corresponding quantity of more dilute alkali is added) and the mixture left for 10 minutes at the ordinary temperature. A small excess of acid is now added, and the excess of acid titrated back with the alkali solution.

Monin (*Rev. Gen. Mat. Col.*, 1910, **14**, 279) also recognised that the

boiling method gives variable results and concluded, on erroneous grounds, that lactic anhydride does not exist in lactic acid solution. He also recommended a new method of estimating lactic acid, but as shown by Besson (*Chem. Zeit.*, 1911, **35,** 26) it is quite worthless and gives variable and always low results, owing to the volatility of lactic acid under the conditions prescribed.

Klaproth (*Chem. Zeit.*, 1911, **35,** 1026) points out that Besson's method gives slightly different results according as 1 or 3 c.c. of alkali is taken. It is also stated to be safe to warm gently after adding the excess of alkali and the following method has been agreed upon by buyers and sellers in Germany for testing the commercial product.

Lactic Acid Method, 1911.—1 grm. of the acid is weighed out in a small beaker (100 to 150 c.c. capacity), diluted with 20 c.c. of water and titrated with N-sodium hydroxide, as free as possible from carbonate, until a permanent pink colouration is obtained. An excess of exactly 1 c.c. of alkali is then added in the case of lactic acids of medium concentration (43.5 to 50% by weight) or 3 c.c. of N-alkali in the case of high percentage acids and the solution warmed on the boiling water-bath for 5 minutes. N-sulphuric acid is then added until the pink colour disappears and an excess of 1 c.c. of acid added; after warming 2 minutes on the boiling water-bath the solution is titrated with the N-alkali solution. The number of c.c. of N-sodium hydroxide used multiplied by 9 gives the percentage by weight of *free acid* (that is lactic acid + 1/2 lactic anhydride), while the total quantity of alkali used in c.c. diminished by the number of c.c. of N-acid, also multiplied by 9 gives the *total acid.*

Example:

1. Titration with N-NaOH: used 4.72 c.c. "Free-acid"$= 4.72 \times 9$ $= 42.68\%$.

Then heated 5 minutes with an excess of 1 c.c. N-NaOH.

2. Titration with N-H_2SO_4: used 0.81 c.c.

Heated on water-bath 2 minutes with excess of 1 c.c. N-H_2SO_4.

3. Final titration with N-NaOH. Used 0.92 c.c.

Total acid calculation:

$$4.72 + 1.0 + 0.92 = 6.64 \text{ c.c. NaOH}$$
$$0.81 + 1.0 \qquad = 1.81 \text{ c.c. } H_2SO_4$$
$$\text{Difference} = 4.83 \text{ c.c.}$$
$$\text{Total acid} = 4.83 \times 9 = 43.47\%$$

The new method gives results lower by 0.7 to 1.5% than the old method. In the case of concentrated acids the difference may be as great as 2.5%.

Tanners usually purchase lactic acid on the basis of free acid only and maintain that the anhydride has no deliming value. But for other purposes it is purchased on the basis of total acid and anhydride.

The method of Ulzer and Seidell (*Monatsh.*, 1897, **18**, 138) is sometimes used for estimating lactic acid in the commercial article. 10 grm. of lactic acid solution is diluted to 1 litre. 100 c.c. of the dilute solution is mixed with a quantity of concentrated potassium hydroxide solution representing 3 grm. KOH, and a 5% solution of potassium permanganate gradually added, shaking constantly, until the solution, which at first is green, becomes violet. The mixture is heated to boiling, when it must remain violet; after cooling, hydrogen peroxide is added until the colour is destroyed. The solution is again boiled, filtered, the precipitate thoroughly washed and the oxalic acid formed by the oxidation is precipitated as calcium oxalate after acidifying with acetic acid. The calcium oxalate is collected, ignited and weighed as calcium oxide. It is assumed that oxidation occurs according to the equation $2C_3H_6O_3 + 5O_2 = 2C_2H_2O_4 + 4H_2O, + 2CO_2$, so that $CaO = C_3H_6O_3$. The method returns lactic anhydride as lactic acid, and oxalic acid or substances yielding oxalic acid on oxidation are also counted as lactic acid.

C. F. Boehringer and Sohn of Nieder-Ingleheim a. Rh., one of the principal German manufacturers of lactic acid, point out (*Färberzeit.*, 1906, **17**, 167) that as commercial lactic acid is frequently adulterated with mineral acids, the tests for sulphuric acid, hydrochloric acid, sulphates and chlorides should always be carried out. Iron should be tested for by potassium ferrocyanide. The guarantee of strength should always refer to weight (grm. per 100 grm.) not to volume as the density is greatly altered by impurities such as sugars, dextrin.

Impurities in Commercial Lactic Acid.

Besides water and lactic anhydride, commercial lactic acid is liable to contain the following impurities:

Inorganic matters, left on igniting the substance. *Sulphuric acid* and *sulphates* will be indicated on adding barium chloride to the 10% aqueous solution of the original substance; *chlorides* by silver nitrate; salts of

calcium by ammonium oxalate; *zinc, lead,* and *iron* by diluting the liquid, nearly neutralising with ammonia, and passing sulphuretted hydrogen.

Foreign Organic Acids.—Of these the presence of *oxalic* or *tartaric acid* will be indicated by the formation of a precipitate on adding lime-water to alkaline reaction, and *citric acid* by precipitation occurring on boiling the liquid so obtained. *Acetic* and *butyric acids* may be recognized by their respective odours on gently heating the liquid; or more certainly by the production of the fragrant odours of their respective ethyl ethers on heating the sample with alcohol and strong sulphuric acid. Ethyl lactate boils at a high temperature and has very little odour. The absence of malic acid and glycollic acid is shown (in the absence of sulphuric acid) by the 10% aqueous solution not producing a turbidity with lead acetate.

Sarcolactic acid may be detected by the formation of a blue *precipitate* on adding cupric sulphate to the 10% aqueous solution of the substance.

Neutral organic matters may, in general, be detected by the production of a brown colour on mixing the sample with an equal measure of cold concentrated sulphuric acid. *Glycerol* may be detected by treating the sample with a slight excess of zinc oxide and a little water, evaporating to dryness at 100°, and treating the residue with ether-alcohol. On evaporation of the solution, glycerol will be left as a sweet syrupy liquid. On treating the residue left undissolved by ether-alcohol with alcohol alone, *cane-sugar* and *dextrose* will be dissolved. Dextrose and other impurities will also be recognised by the formation of a red or yellow precipitate on heating the neutralised acid with Fehling's solution.

Lactic acid should not be materially coloured when heated with a strong solution of alkali hydroxide, and should be wholly soluble in ether.

Requirements.—*United States Pharmacopœia.* The following are the requirements of the United States Pharmacopœia (8th Revision).

Sp. gr.: about 1.206 at 25°.

Freely miscible with water, alcohol, or ether; insoluble in chloroform, petroleum benzin, or carbon disulphide.

Lactic acid is not vaporised by a heat below 160°; at a higher temperature it emits inflammable vapors, and is finally dissipated; 5 grm.,

after combustion, should not leave more than 0.05 grm. of fixed residue.

Lactic acid has an acid reaction upon blue litmus-paper.

On adding some potassium permanganate to a mixture of equal volumes of lactic and sulphuric acids, and gently heating, the odour of aldehyde will become perceptible.

10 c.c. of a solution of the acid in distilled water (1 in 100) should not be rendered opalescent by 1 c.c. of silver nitrate T.S. (limit of *chloride*).

10 c.c. of an aqueous solution (1 in 20) should remain unaffected by the addition of 1 c.c. barium chloride T.S. (absence of *sulphate*), or by 1 c.c. of copper sulphate T.S. (absence of *sarcolactic acid*), nor should it respond to the time-limit test for *heavy metals*.

On adding a few drops of lactic acid to 10 c.c. of hot alkaline cupric tartrate V.S., no red precipitate should be formed (absence of *sugars*).

On warming lactic acid the odour of rancid fat should not be noticeable (absence of *butyric* and *other fatty acids*).

If a small portion of the acid be heated on a water-bath with an excess of zinc carbonate, the mixture dried at 100° and then extracted with absolute alcohol, upon evaporation of the latter no sweet residue should remain (absence of *glycerine*).

On carefully pouring lactic acid upon an equal volume of colourless concentrated sulphuric acid contained in a clean test-tube, and keeping the temperature at or below 15° (59° F.), no dark-coloured zone should develop at the line of contact upon standing for 15 minutes (absence of more than traces of *organic impurities*).

If 5 grm. of lactic acid be diluted with water to measure 50 c.c., then 44.7 c.c. of this solution should require for complete neutralisation at boiling temperature not less than 37.5 c.c. of normal potassium hydroxide V.S. (each c.c. corresponding to 2% of absolute lactic acid), phenolphthaleïn T.S. being used as indicator.

British Pharmacopœia Requirements (1898).—Lactic acid is defined as "a liquid containing 75% of hydrogen lactate with 25% of water." This definition takes no account of the lactic anhydride invariably present. The sp. gr. of the British Pharmacopœia solution is 1.21. The solution has to satisfy the following tests: Not more than 0.5% of solid matter should remain after ignition. Each grm. should require for neutralisation 8.3 c.c. of *N* solution of sodium hydroxide. It should yield no characteristic reaction with the tests for lead, copper, arsenic, iron, aluminium, chlorides, citrates, oxalates, phosphates,

sulphates or tartrates. The acid when diluted gives no precipitate with solution of copper sulphate (absence *of sarcolactic acid*) and none or only the slightest trace with excess of solution of potassio-cupric-tartrate, even after prolonged boiling (*absence of more than traces of grape, cane- and milk-sugar*). The mixture obtained by heating lactic acid with excess of zinc carbonate and evaporating to dryness, should not, when exhausted with absolute alcohol, and the latter evaporated, yield any sweet residue (*absence of glycerol*). Gently warmed there should be no rancid odour (*absence of fatty acids*). Carefully poured upon an equal volume of sulphuric acid contained in a test-tube little or no darkening should ensue (*absence of organic impurity*). No turbidity, either permanent or transient, should be produced when the acid is added to twice its volume of ether (*absence of gum, sugar, mannitol, calcium phosphate*). It should give no precipitate with a solution of lead subacetate (*absence of malic and sulphuric acids*).

Salts of Lactic Acid.

Metallic Lactates are all more or less soluble in water, but usually dissolve only sparingly in the cold. They are all insoluble in ether. The sarcolactates are usually more soluble than the salts of ordinary lactic acid, and are lævorotatory.

Calcium Lactate, $Ca(C_3H_5O_3)_2,5H_2O$, is obtained in crystals when lactic acid is neutralised with lime or chalk, and the liquid concentrated. It crystallises in small white mammillated tufts, which under the microscope appear as delicate rhombic needles, some of which look like bundles bound in the centre. From acid solutions, a so-called *acid lactate* of calcium (a compound of calcium lactate with lactic acid) crystallises in radiating trimetric needles or fibrous masses. One part of calcium lactate dissolves in 9.5 parts of cold water, and in all proportions in boiling water or alcohol (compare calcium sarcolactate, page 450). When calcium lactate is heated, it readily parts with its water of crystallisation, and at 250 to 260° is converted into a tumefied mass containing calcium dilactylate, $O[CHMe.CO_2]_2Ca$, from which absolute alcohol dissolves out any unaltered lactate, leaving the dilactylate as a sparingly soluble residue. The corresponding dilactylic acid melts at 106° (Jungfleisch and Godchot, *Compt. Rend.*, 1907, **144**, 979).

Ferrous Lactate, $Fe(C_3H_5O_3)_2,3H_2O$, crystallises in light yellow

needles, soluble in 48 parts of cold or 12 parts of boiling water. The dry salt is permanent, but the solution rapidly oxidises. Ferrous lactate is not unfrequently adulterated, the substances used for the purpose being dried ferrous sulphate, milk-sugar, and starch. A sample of "lactate of iron," examined by M. Peltier, contained 25% of ferrous sulphate and 75% of milk-sugar. *Ferrous sulphate* can be readily detected by the copious precipitate produced on treating the solution of the sample with barium chloride. *Milk-sugar* may be detected by rendering the solution alkaline by soda, passing hydrogen sulphide to precipitate the iron, filtering, adding Fehling's solution to the filtrate, filtering rapidly in the cold from the copper sulphide, and heating the filtrate, when a yellow or red precipitate of cuprous oxide will be formed if milk-sugar be present. *Starch* may be detected in the portion of the sample insoluble in cold water, by the blue colour produced on addition of solution of iodine.

Lead Lactate, $Pb(C_3H_5O_3)_2$, is freely soluble in water, sparingly soluble in cold, but readily in hot alcohol, and slightly soluble in ether. It does not crystallise, but forms a gummy mass on evaporation. (*Glycerate* of lead is but slightly soluble in cold water.) By adding lead acetate and alcoholic ammonia, lactic acid is completely precipitated as a compound containing $3PbO,2C_3H_6O_3$ (compare page 433).

Zinc Lactate, $Zn(C_3H_5O_3)_2,3H_2O$, crystallises from concentrated solutions in shining crusts, or from dilute solutions in four-sided prismatic needles, soluble in 53 parts of water at 15°, or 6 of boiling water, and insoluble in alcohol (compare zinc sarcolactate, page 450). The water of crystallisation is lost rapidly at 100°, and above 210° the salt decomposes.

Magnesium Lactate, $Mg(C_3H_5O_3)_2,3H_2O$, forms a white crystalline powder or prismatic needles and is soluble in 23.8 parts of water at the ordinary temperature. It is used to some extent in pharmacy. *Bismuth Lactate* also finds use in medicine.

"*Lactolin*," which has found considerable application in wool dyeing, especially as an assistant to the dichromate mordant, is *acid potassium lactate*, or an equimolecular mixture of lactic acid and normal potassium lactate (Kielmayer, *Färberzeit.*, 1899, **10**, 17 and 33; Dreher, *ibid.*, 68).

"*Antimonin*" is calcium antimony lactate (Düring, *Färb. Zeit.*, 1900, **11**, 319. Chem. Werke Schuster und Wilhelmy, D. R. P. 216158). *Sodium antimony lactate* and *sodium calcium antimony lactate* have been patented by von Heyden (D. R. P. 184202). They

are used as tannin-fixing mordants. Compounds of titanic acid with lactic acid are used in the leather industry (Dreher, Eng. patents, 9556, 1901; 22629, 1901; 23188, 1901; 9556, 1902).

Glycerol mono- and di-lactates (Kalle and Co., D. R. P. 21697, 1910) are employed therapeutically as lactic acid substitutes.

Estimation of Lactic Acid in Lactates.

For this purpose Paessler's method (*Collegium*, 1907, 388 and 396) is generally used. It gives good results in some cases when other methods are either useless or too laborious. It depends on the oxidation of lactic acid to acetic acid and carbon dioxide in sulphuric acid solution by a standard solution of potassium dichromate solution according to the equation

$$3C_3H_6O_3 + 2 K_2Cr_2O_7 + 8H_2SO_4 =$$
$$3 K_2SO_4 + 2Cr_2(SO_4)_3 + 3C_2H_4O_2 + 3CO_2 + 11H_2O.$$
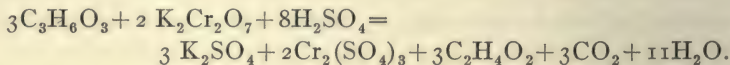
An accurately weighed portion of the material (about 0.4 grm.) or an aliquot proportion of a suitably diluted solution, is boiled with 10 c.c. of 10% sulphuric acid and 25 c.c. of $N/2$ potassium dichromate solution, in an Erlenmeyer flask fitted with a reflux condenser. The excess of dichromate solution remaining is then titrated in the ordinary way with $N/10$ sodium thiosulphate solution after adding 10 c.c. of 10% potassium iodide solution. Starch paste is used as final indicator in the usual manner. 1 c.c. of $N/2$ potassium dichromate = 0.01127 grm. of lactic acid. Volatile matter which reduces chromate solution should be removed by repeated evaporation before the estimation. Sugar, dextrin, glycerol or similar materials which reduce chromate solution and cannot be removed by volatilisation, render the method useless. Good results are obtained by this method with such salts as calcium antimony lactate, and sodium antimony lactate, containing more or less excess of lactic acid. In such cases the antimony is first precipitated as antimony sulphide by hydrogen sulphide, the solution filtered and any excess of hydrogen sulphide removed by boiling. Lactic anhydride is not oxidised by the above treatment and if present must first be converted into lactate by heating with a slight excess of alkali.

Active Lactic Acids.

By adopting the principle previously applied by Pasteur to the preparation of dextrorotatory and lævorotatory tartaric acids from

inactive racemic acid, T. Purdie (*Jour. Chem. Soc.*, **61**, 754) found that ordinary inactive lactic acid could be decomposed into two oppositely active lactic acids by fractional crystallisation of the strychnine salt, that of the lævorotatory acid being the less soluble. The two free acids exhibit equal and opposite optical activities to those of their salts. The zinc salts of each of the optically active acids crystallise with $2H_2O$. On mixing aqueous solutions of equal quantities of the two zinc salts, and stirring the liquid, inactive zinc lactate containing $3H_2O$ separates. The two optically active modifications of lactic acid present a very close resemblance to ordinary lactic acid and to each other. The anhydride and salts of dextrolactic acid are lævorotatory, while the anhydride and salts of lævolactic acid are dextrorotatory. On heating, both active modifications yield the same lactide, and when this takes up the elements of water, it yields ordinary inactive lactic acid. Lævolactic acid was stated by Lewkowitsch (*Ber.*, 1883, **16**, 2720) to be produced by growing the mould *Penicillium glaucum* in a solution of ammonium lactate. A repetition of this experiment by Linossier (*Bull. Soc. Chem.*, [3], **6**, 10) gave an opposite result, the residual lactic acid yielding lævorotatory salts. F. Schardinger (*Monatsh.*, **11**, 551) obtained lævolactic acid by the fermentation of cane-sugar by the *Bacillus acidi lævolactici*, which closely resembles the ordinary lactic acid bacillus in appearance, but has considerably greater fermentative power.

Dextro-Lactic Acid. Sarcolactic Acid. Paralactic Acid.

This acid is obtainable in the manner above described from inactive lactic acid. It occurs naturally in the juices of muscular tissue, in bile, and in the urine of persons poisoned by phosphorus. It is also obtained in numerous impure fermentations (*e.g.*, of dextrin, dextrose, cane-sugar, milk-sugar, etc.). Frankland and Macgregor obtained sarcolactic acid by the interrupted bacterial fermentation of ordinary inactive calcium lactate (*Jour. Chem. Soc.*, 1893, **63**, 1028).

It is generally present in organs undergoing pathological change. During muscular activity the proportion of lactic acid present is increased.

Sarcolactic acid presents the closest resemblance to ordinary lactic acid. The most tangible distinctions are: (1) The dextrorotation of free sarcolactic acid, and the lævorotation of its anhydride and salts; ordinary lactic acid, when pure, both in the free state and in the form

of salts, being optically inactive. Fermentation lactic acid frequently contains more or less of the active forms. (2) The solubility and amount of water of crystallisation in the calcium and zinc salts. (3) Ordinary lactic acid yields a deep blue liquid on addition of cupric sulphate, while sarcolactic acid is almost completely precipitated by that reagent.

Sarcolactic acid is conveniently prepared by dissolving extract of meat in 4 parts of water, and adding to the solution three times its volume of methylated spirit. The filtered liquid is evaporated to a syrup, which is again treated with alcohol, filtered, evaporated, acidified with sulphuric acid, and extracted with ether. The acid thus obtained on evaporation of the ether may be purified by conversion into the zinc salt.

When pure, d-lactic acid forms flattened, radiating prisms, melts at 25 to 26° and is very hygroscopic. In 10.5% aqueous solution $[\alpha]_D^{15} = +3.82$, in 2.5% solution $[\alpha]_D^{15} = +2.67$.

On heating, sarcolactic acid yields a lævorotatory anhydride, the solution of which is lævorotatory; but this active anhydride and the lactide formed on further heating are hydrolysed by water to a solution of ordinary inactive lactic acid.

Calcium sarcolactate, $Ca(C_3H_5O_3)_2 + 4H_2O$, is soluble in about 12 parts of cold water. The solution is lævorotatory, the value of $[\alpha]_D$ for a solution containing 5.35% of the anhydrous salt being stated by Wislicenus at $-5.48°$.

Zinc sarcolactate, $Zn(C_3H_5O_3)_2 + 2H_2O$, crystallises in slender needles which lose their water slowly at 100°, and give off empyreumatic vapours below 150°. The salt is soluble in 17.5 parts of water at 15°, but only sparingly soluble in cold alcohol (distinction from ethylene-lactic acid). The aqueous solution of zinc sarcolactate is lævorotatory, the following being the values for $[\alpha]_D$ found by Wislicenus for solutions of various strengths:

$c = 16.05$[1]	$[\alpha]_D = -6.36$
11.01[1]	-6.36
7.47	-6.83
6.13	-7.41
5.26	-7.60

Lævolactic Acid.—This as stated above was first obtained by Schardinger. Friedländer's pneumonia bacillus produces l-lactic

[1] Supersaturated.

acid from a number of carbohydrates, disaccharides and glycerol.

l-*Lactic acid* melts at 26 to 27°. Its salts have the same composition and solubility as the salts of *d*-lactic acid; they are, however, dextro-rotatory, the dextrorotation being equal in magnitude, under the same conditions, to the lævorotation of the *d*-salts.

Lactic Acid in Organic Tissues and Extracts.

The detection and estimation of lactic acid in organic tissues, organs, etc., can only be carried out after removing fats and proteins. The material is extracted with water and albumin removed from the extract by coagulation, by boiling after adding a little dilute sulphuric acid. Afterwards the liquid is accurately neutralised by baryta at the boil, and, after filtering, the liquid is evaporated to a syrup. This is precipitated with absolute alcohol and the precipitate thoroughly extracted with alcohol. From the united alcohol extracts, the alcohol is distilled off, and the neutral residue shaken with ether to remove fats. The residue is then taken up with water, phosphoric acid added, and again shaken with ether, which now removes the lactic acid From the united ethereal extracts (the extraction is best carried out as described under the Buchner and Meisenheimer method, page 437) the ether is distilled off, the residue is dissolved in water and carefully warmed on the water bath to remove ether and volatile acids. The filtered solution is then boiled with zinc carbonate to obtain a solution of zinc lactate which is evaporated until crystallisation begins, 15 c.c. of alcohol is added and the solution is then left in a desiccator over sulphuric acid, and treated as in Buchner and Meisenheimer's method. The resulting crystalline salt should be analysed (water and zinc oxide) to make certain of its identity, and the optical rota-tion observed, so as to distinguish between the different forms of lactic acid likely to occur.

Ethylene Lactic Acid. Hydracrylic Acid.

β-Hydroxypropionic Acid.

$$C_3H_6O_3 \text{ or } OH.CH_2.CH_2.COOH$$

This compound is distinguished from the other modifications of lactic acid by the fact that it yields no trace of lactide when heated, being resolved, almost without residue, into water and acrylic acid,

$CH_2:CH.CO_2H$. The same decomposition occurs on heating it with sulphuric acid diluted with an equal weight of water. On the other hand, when acrylic acid is heated to 100° with excess of sodium hydroxide solution, hydracrylic acid is reproduced. Hydracrylic acid has only been obtained by synthetical means,[1] its formation from β-iodopropionic acid by boiling with water or heating with moist silver oxide being the most available reaction.

On oxidation with nitric acid, hydracrylic acid yields carbon dioxide and oxalic acid. With chromic acid mixture, the former is the sole product of the action.

The sodium and calcium salts of hydracrylic acid melt without change at about 140 to 145°, but at a higher temperature they lose water and are converted into acrylates. *Zinc hydracrylate*, $(C_3H_5O_3)_2$ $Zn,4H_2O$, crystallises in large shining prisms, soluble in an equal weight of cold water (100 parts of salt dissolve in 89 parts of water at 16.5°). The salt is also soluble in alcohol, which precipitates zinc lactate and sarcolactate from their aqueous solutions.

[1] Wislicenus obtained from flesh, together with sarcolactic acid, an acid which he supposed to be ethylene-lactic acid, but it was shown by Siegfried (*Ber.*, 1889, **22,** 2713) that this was really acetyl-lactic acid.

CYANOGEN AND ITS DERIVATIVES.

BY HERBERT PHILIPP.

The term "cyanogen derivatives" covers a group of substances derived from "cyanogen," C_2N_2.

Cyanogen itself is a gaseous product and in its reactions shows some resemblance to the halogens. Cyanogen can be obtained in a free state, and enters into direct combination with metals and also with hydrogen. The hydrogen compound, hydrocyanic acid, is a product with distinct acid properties, showing analogy to the halogen acids. The hydrogen in hydrocyanic acid can be replaced by a metallic element and forms salts, closely resembling in many ways the halogen salts. Silver cyanide, for instance, is insoluble in water and dilute acids, is of a cheese-like consistency and soluble in ammonium hydroxide, thus showing a great similarity to silver chloride.

The metallic cyanides are a very important class of salts and have an extensive industrial application. Most of the metals form compounds with cyanides, and many of the metallic cyanides combine with each other, forming often very complex salts.

The double salts formed, by the dissolving of insoluble cyanide salts of the heavy metals, by cyanide salts of the light metals, fall into two classes. One class consists of those double salts which are decomposed by dilute mineral acids into the insoluble cyanide of the heavy metal and free hydrocyanic acid, as for instance $KCN,AgCN$; $2KCN,Ni(CN)_2$. The other class does not liberate hydrocyanic acid, but behaves as salts of characteristic acids. To this latter class belong potassium ferrocyanide $(K_4Fe(CN)_6)$ and potassium ferricyanide $(K_3Fe(CN)_6)$, which form, by the addition of dilute mineral acids, the acids hydrogen ferrocyanide and hydrogen ferricyanide. Many salts of the latter class are not affected at all by acids, as for example, Prussian blue, which is, however, converted by alkali hydroxide into ferric hydroxide and alkali ferrocyanide.

The cyanamide compounds, which are closely related to the cyanide

compounds, form a new important industry owing to their recent application as an artificial fertilzer. Their probable use in the future as a raw material in the production of ammonia compounds forms the basis of several patents.

All cyanide compounds, irrespective of in what form the cyanogen may be combined, will yield up their total nitrogen in the form of ammonia when they are heated to a high temperature with an excess of alkali or soda-lime. If the compounds are heated with concentrated sulphuric acid, as in the Kjeldahl's process, all the nitrogen is converted into ammonia.

Cyanogen.

Cyanogen, sometimes called dicyanogen, is composed of carbon and nitrogen. Cyanogen occurs free in blast furnace gas, coal gas and between carbon electrodes in a nitrogen atmosphere.

Cyanogen is considered a nitrile of oxalic acid, because when ammonium oxalate is treated with a dehydrating agent, as for instance phosphorus pentoxide, cyanogen is formed. Inversely if cyanogen is dissolved in hydrochloric acid, it takes up four molecules of water and forms ammonium oxalate.

Henry (*Ber.*, 1869, **2**, 307) obtained cyanogen by taking five molecules of carefully dried oxamide, mixing thoroughly with two molecules of phosphorus pentoxide, and heating the mixture in a retort.

These reactions are a proof that cyanogen is the nitrile of oxalic acid (ethane dinitrile), having thus the structural formula

$$C\equiv N$$
$$|$$
$$C\equiv N.$$

Cyanogen, C_2N_2, is generally prepared by heating desiccated mercuric cyanide in a retort to a high temperature. (Gay-Lussac, *Gilberts Annalen*, 1816, **53**, 139). The prepared cyanogen gas is collected over mercury, leaving in the retort, besides metallic mercury, a brown solid product, paracyanogen, which is converted into cyanogen at a high temperature. Kemp (*J. f. prakt. Chem.*, 1881, **31**, 63) prepares cyanogen by heating a mixture of potassium ferrocyanide with mercuric chloride.

A very good method for preparing cyanogen is described by Jacque-

min (*Compt. Rend.*, 1885, **100,** 1005). A concentrated aqueous solution of one part potassium cyanide is poured into a solution consisting of two parts copper sulphate in four parts of water. The temperature rises to about 40° and the solution has finally to be heated. First cupric cyanide is precipitated, which breaks up into cyanogen and cuprous cyanide:

$$2CuSO_4 + 4KCN = Cu_2(CN)_2 + C_2N_2 + 2K_2SO_4.$$
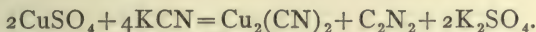
The cyanogen in the cuprous cyanide formed can be liberated by further treatment with ferric chloride (l.c.).

Cyanogen is a colourless gas having a pungent odour, smelling similar to bitter almonds. It is very stable at high temperatures, but in the air it burns with a characteristic red violet flame. Its sp. gr., according to Gay-Lussac, is 1.8064 (air=1), according to Thomsen 1.80395.

1 volume water at 20° dissolves 4.5 volumes cyanogen.

1 volume alcohol dissolves 23 volumes cyanogen.

1 volume glacial acetic acid dissolves 80 volumes cyanogen.

Cyanogen liquefies if it is cooled to −20.7° or compressed to 3.3 atmospheres at 15°. The critical temperature and pressure are $t_c = 128.3$; $p_c = 59.6$ (Cordoso and Baume, *Compt. Rend.*, 1911, **151,** 141). Liquid cyanogen has a sp. gr. 0.866 at 17.2° and does not conduct electricity; by further cooling it crystallises to a transparent crystalline mass, which melts at −34.4°.

Cyanogen, either gaseous or liquid, when dry and pure can be kept for considerable time without decomposing. Cyanogen gas in the presence of oxygen, when heated or passed through the electric arc, slowly decomposes. Equal volumes of cyanogen and oxygen explode in the eudiometer with great vehemence. Dixon (*J. of Gaslighting,* 1896, **68,** 460 or *Chem. News,* 1897, **73,** 138) states that carbon monoxide and nitrogen are formed. If an excess of oxygen is used carbon dioxide is formed as well. The intensiveness of the explosion decreases by increasing the amount of oxygen; the explosion is, however, accompanied by a brilliant flame.

Aqueous and alcoholic solutions of cyanogen are not very stable, decomposing slowly to azulminic acid, a brown flocculent precipitate. The solution is found to contain oxalic acid, hydrocyanic acid, carbon dioxide, ammonia and urea. Zettle (*Monatshefte f. Chem.,* 1893, **14,** 223) states that by the addition of small quantities of mineral acids the cyanogen solutions are made more stable.

In crude coal gas cyanogen and its compounds are present in small quantities. Besides free cyanogen hydrocyanic acid, ammonium cyanide, methyl cyanide, thiocyanic acid, thiocyanogen and ammonium thiocyanate are present. There is no method at present by which these compounds can be quantitatively separated. A small part of the cyanogen compounds dissolves in the water of the drip pots, coolers and scrubbers (Pfeiffer, *J. f. Gasbelch*, 1898, **70,** 69). Most of the cyanogen and cyanogen compounds are, however, taken up by the ferrous oxide in the purifiers.

A good qualitative method for testing whether cyanogen is present in the purified coal gas is the isopurpuric acid reaction suggested by Kunz-Krause (*Zeit. f. angew. Chem.*, 1901, **14,** 652). 2 c.c. of a freshly prepared concentrated aqueous solution of picric acid (1:86) are mixed with 18 c.c. alcohol and 5 c.c. potassium hydroxide solution (15%). The gas to be tested is passed through this solution, and if cyanogen or hydrocyanic acid are present a red-brown colouration, due to the formation of isopurpuric acid, is produced.

As already stated, no quantitative method exists whereby the amount of free cyanogen alone can be estimated, but a method described by Drehschmidt (*J. f. Gasbelch.*, 1892, **64,** 268) is generally used for the estimation of the total cyanogen either in crude coal gas or the purified gas.

The gas to be tested is passed through two Drehschmidt gas wash cylinders, especially designed for this purpose. The first cylinder contains 15 c.c. of a ferrous sulphate solution and 15 c.c. of a 25% potassium hydroxide solution. (The author uses sodium hydroxide instead of potassium hydroxide without affecting the results). The second cylinder contains 10 c.c. of a 10% ferrous sulphate solution, 5 c.c. of a 25% potassium hydroxide and 20 c.c. water. The gas wash cylinders are connected in series and the gas allowed to pass through. 100 litres in all are used, occupying from one and a quarter to one and a half hours in its passage. If the pressure of the gas is not enough to pass the gas through the gas wash cylinders, then the second gas wash cylinder is connected with a suction pump or aspirator bottle. After the gas has passed through the wash cylinders, the contents of the cylinders are washed into a 300 c.c. volumetric flask and the free alkali is approximately neutralised by sulphuric acid, the amount of sulphuric acid being determined by a previous test. 2 grm. ammonium sulphate, 30 grm. mercuric oxide and a few drops of ammonia

are now added to the contents of the flask, and boiled gently for about a quarter of an hour to allow the cyanogen to be converted to mercuric cyanide. The flask is filled to the mark and 2.6 c.c. excess water is added; this is to correspond to the volume of the precipitate, and the solution is then filtered through a dry filter. 250 c.c. of the filtrate are put in a 300 c.c. volumetric flask, to which is added 10 c.c. ammonium hydroxide (sp. gr. 0.91), and then 7 grm. zinc dust. It is very important that the zinc dust should be free from chlorine compounds, which is accomplished by previously washing the zinc dust with ammonium hydroxide. The precipitation of the mercury is accelerated by frequently shaking the flask. All the cyanogen has now been converted to ammonium cyanide. Now 2 c.c. of a 30% potassium hydroxide solution (free from chlorine) is added to prevent any hydrocyanic acid from volatilising. The flask is filled to the mark, 1 c.c. more water is added to compensate for the volume of the zinc dust. The contents of the flask are filtered, after mixing, through a dry filter. 150 c.c. of the filtrate are taken and put in a 500 c.c. volumetric flask to which 35 c.c. $N/$10 silver nitrate solution are added. 25 c.c. of a 10% nitric acid solution are added to the contents of the flask and all the silver cyanide is precipitated. The flask is filled to the mark, shaken and filtered through a dry folded filter. 250 c.c. of the filtrate are taken and the excess of silver estimated by Volhard's method with ammonium thiocyanate solution. A few c.c. of a cold saturated solution of iron ammonium alum, acidified with nitric acid, are added, and $N/$10 ammonium thiocyanate run in until a permanent red colour is obtained. The number of c.c. used subtracted from the 35 c.c. $N/$10 silver nitrate used, gives the amount of silver used to combine with the cyanogen present.

The amount of cyanogen present equals the number of c.c. $N/$10 silver nitrate used mutiplied by 0.002601 × 12/5, or number of c.c. $N/$10 silver nitrate used × 0.006242, giving grm. total cyanogen in 100 litre gas. Or if n=number of c.c. decinormal silver nitrate used, then

$$n \times 6.242 = \text{grm. total cyanogen in 100 cb. m. gas.}$$
$$n \times 1.7675 = \text{grm. total cyanogen in 1000 cu. ft. gas.}$$
$$n \times 27.2767 = \text{grains total cyanogen in 1000 cu. ft. gas.}$$

Leybold's method (*J. f. Gasbelch.*, 1890, **72**, 336 and 427) is occasionally used for the estimation of cyanogen in gas. The sample is taken in the same way as in Drehschmidt's method, by allowing 100 litres gas

to pass through gas wash cylinders in which ferrous hydroxide is held suspended in an alkaline solution (see previous method). The contents of the two gas wash cylinders are washed into an Erlenmeyer flask and boiled for about 10 to 15 minutes. This is to convert all cyanogen into ferrocyanide. Precaution should be taken not to boil too long, as there is a possibility of forming thiocyanate, sulphur being generally present. The contents of the flask are allowed to settle, and are filtered cold through a folded filter. The iron sulphide and hydroxide remain on the filter and must be washed out until the wash water gives no blue reaction with acidified iron chloride solution. The filtrate is put in a 500 c.c. volumetric flask and filled up to the mark. After thoroughly shaking the flask an aliquot part of the solution is taken, the quantity varying according to whether the sample is taken before or after the purifiers. If the sample is taken before the purifiers then 100 c.c. of the solution are used, if after the purifiers 200 c.c. of the solution are taken. The 100 c.c. taken are acidified with hydrochloric acid and an excess of a ferric chloride solution (10%) is added, so that all the ferrocyanide present is precipitated as Prussian blue. As soon as the Prussian blue has settled, the solution is poured through a folded filter, finally washing the whole precipitate on to the filter. The precipitate has to be washed with small quantities of water till it is free from ferric chloride, which can be detected by the wash water losing its yellow colour. The filter with the precipitate is placed in a wide neck Erlenmeyer flask, and stirred with a dilute sodium hydroxide solution. The contents of the Erlenmeyer flask are diluted and filtered, washing the residue with hot water, till the wash water shows no blue reaction with acidified ferric chloride solution.

The filtrate is evaporated, preferably in a platinum dish, not quite to dryness, leaving 20 to 30 c.c. in the dish. The contents of the dish are strongly acidified with dilute sulphuric acid (10%) and the dish is now placed on a hot plate or sand-bath, and the excess of sulphuric acid is evaporated, finally igniting over direct flame. The evaporation and treatment with sulphuric acid should be done in a well ventilated hood. The residue is taken up with about 100 c.c dilute sulphuric acid (10%) and washed into a 250 to 300 c.c. flask. Should any iron oxide remain fast on the side of the dish, it should be dissolved in as few drops of hydrochloric acid as possible. The hydrochloric acid is driven off by adding a little dilute sulphuric acid and evaporating till

the sulphuric acid begins to fume; a little water is carefully added to the dish and the contents washed over to the main portion in the flask.

8 to 10 grm. pure zinc are put in the flask to reduce all the iron to a ferrous state. It has been recommended to add also a few drops of a copper sulphate solution, which forms an electrochemical chain and hastens the reduction of the ferric iron. The reduction is completed after about 3 hours heating on the water-bath, and it is best to provide the flask with a Bunsen valve, to prevent currents of air entering the flask. After 3 hours on the water-bath, a drop taken out of the flask should show no red colour with a potassium thiocyanate solution, otherwise the reduction is not completed.

After the reduction is complete, the flask is allowed to cool and the liquid filtered quickly through a folded or suction filter, immediately washing the filter with about 100 c.c. of water. The filtrate is strongly diluted in an Erlenmeyer flask and is at once titrated with $N/10$ potassium permanganate solution. If n represents the number of c.c. decinormal potassium permanganate solution used, then:

$n \times$ 0.015606 $\times 5 =$ grm. total cyanogen in 100 litre gas.
$n \times$ 78.03 $=$ grm. total cyanogen in 100 cb. m. gas.
$n \times$ 22.096 $=$ grm. total cyanogen in 1,000 cb. ft. gas.
$n \times 340.994 =$ grains total cyanogen in 1,000 cb. ft. gas.

In cases where very little cyanogen is in the gas and 200 c.c. of the solution are originally used, the above figures must be divided by 2.

Paracyanogen.

Paracyanogen is formed when the desiccated metal cyanides are heated in a retort; silver cyanide especially forms this polymeric compound of cyanogen, $(CN)_x$. It is also formed by heating azulminic acid, cyanogen chloride or liquid cyanogen to a high temperature.

Paracyanogen is a spongy, brown-black, amorphous mass, which is insoluble in water and alcohol, is not acted on by nitric acid, but is soluble in concentrated sulphuric acid. With potassium or sodium hydroxides it forms the corresponding alkali cyanate.

Paracyanogen heated to 860° in a nitrogen or carbon dioxide atmosphere is totally converted into cyanogen gas. Ignited in a current of hydrogen gas, it forms hydrocyanic acid, ammonia and carbon.

Cyanogen Halides.

Cyanogen unites with the halogens to form compounds with characteristic properties.

Cyanogen Chloride, CNCl, is formed by passing chlorine gas through hydrocyanic acid or by allowing chlorine to react with mercuric cyanide in the dark and at a low temperature.

It is generally prepared by taking a large flask, filled with chlorine gas, in which some moistened pieces of mercuric cyanide are placed, each litre of chlorine requiring 5 grm. mercuric cyanide; it is then allowed to stand in the dark at a cool temperature until the colour of the chlorine gas has disappeared. A little mercury is then added and the whole flask shaken to allow any uncombined chlorine to combine with the mercury. After this the flask is connected with a cooler and slowly heated. The evolved gas, cyanogen chloride, is passed over calcium chloride and condensed in a freezing mixture. Great care must be taken in handling the cyanogen halides as they are all volatile compounds and are extremely poisonous.

Cyanogen chloride is a colourless gas with a pungent odour. It powerfully affects the eyes, has an acrid taste and is very poisonous. It solidifies between -5 and $-6°$, forming transparent prisms and boils between 12 and 15°. Its vapour density is 2.13.

1 volume water dissolves 25 volumes cyanogen chloride.
1 volume ether dissolves 50 volumes cyanogen chloride.
1 volume alcohol dissolves 100 volumes cyanogen chloride.

Cyanogen chloride is decomposed by alkalies into cyanates and chlorides, a similar reaction taking place with water if a cyanogen chloride solution is allowed to stand too long.

Silver nitrate does not precipitate silver chloride nor silver cyanide from cyanogen chloride. Several metallic chlorides form crystalline compounds with cyanogen chloride.

The best method for the estimation of cyanogen chloride consists in the liberation of iodine from potassium iodide in slightly acid solution.

$$CNCl + HCl + 2KI = 2KCl + HCN + I_2.$$
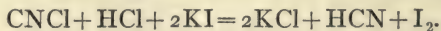
The presence of hydrocyanic acid or alkali cyanide does not affect this reaction. The iodine liberated is titrated with $N/10$ sodium thiosulphate.

Solid cyanogen chloride, $(CN)_3Cl_3$, is a polymer of cyanogen chloride. It forms directly from cyanogen chloride if the latter is stored for a long time, especially when exposed to light.

Solid cyanogen chloride melts at 140° and boils at 190°. It forms monoclinic needles, sp. gr. 1.32. It has an acrid taste and is a power-

ful poison. It has a pungent odour and acts on the eyes. When dilute, the odour is compared to that of the excrement of mice.

Solid cyanogen chloride is slightly soluble in water and is decomposed by boiling.

Cyanogen Bromide, CNBr, is formed similarly to cyanogen chloride. This product is of industrial interest in connection with the "Sulman-Teed gold extraction process" (*J. Soc. Chem. Ind.*, 1897, **16,** 961); full details of how the modified process is carried out at present are published by Williams (*Eng. and Min. J.*, 1908, **85,** 345). It has an advantageous solvent action on ores which contain the precious metals such as tellurides, selenides, etc., when mixed with the cyanide solution.

A very easy method for preparing the cyanogen bromide has been recommended by Dr. Hans Foersterling (private communication), which consists in allowing hydrogen peroxide to react on a slightly acid solution of sodium cyanide and sodium bromide.

$$NaCN + NaBr + H_2SO_4 + H_2O_2 = CNBr + Na_2SO_4 + 2H_2O.$$
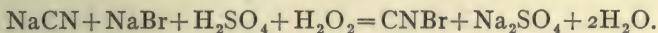
This reaction takes place very quickly, and to obtain good yields concentrated solutions should be used. This same process can be used for forming cyanogen chloride as well as cyanogen iodide.

Cyanogen bromide crystals give a sublimate of delicate prisms which rapidly change to cubes. Cyanogen bromide has a pungent odour, powerfully affects the eyes and is very poisonous. It melts at $52°$ and boils at $61°$. It slowly volatilises from the solid product above $15°$. It is very soluble in water and alcohol.

Alkali hydroxides, also water, decompose cyanogen bromide, forming alkali bromides and cyanates. Alkali cyanides decompose cyanogen bromide by evolving cyanogen gas.

Polymeric cyanogen bromide, $(CN)_3Br_3$, is formed by passing hydrogen bromide through an ethereal solution of cyanogen bromide.

Cyanogen Iodide, CNI, forms more easily and gives better yields than the other cyanogen halides. It is produced in the quantitative estimation of cyanide by Fordos and Gelis' method (page 483), and can also be produced by the methods described under the other cyanogen halides.

Cyanogen iodide crystallises in long transparent needles. It is extremely poisonous, has an odour like that of cyanogen bromide and volatilises at ordinary temperatures, although it has a high boiling-

point, boiling below 100°. It is soluble in alcohol and ether, but only sparingly soluble in water.

When iodine is added to a solution of hydrocyanic acid, a certain amount of cyanogen iodide is formed:

$$HCN + I_2 = HI + CNI,$$
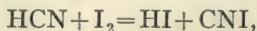
but beyond a certain point the iodine is no longer acted upon. The amount of iodine capable of being converted into cyanogen iodide, the amount of hydrocyanic acid remaining constant, increases both with the temperature and with dilution. Cyanogen iodide is completely decomposed by hydriodic acid, sulphurous acid, hydrogen sulphide, stannous chloride, and other reducing agents, but is as stable as iodic acid toward oxidising agents.

Hydrocyanic Acid and Simple Cyanides.

Simple metallic cyanides are the salts of hydrocyanic acid, also known as prussic acid. Hydrocyanic acid is the nitrile of formic acid, $H-C\equiv N$, inasmuch as it is formed by the distillation of ammonium formate, and reversely transformed into ammonium formate by dissolving in hydrochloric acid. Michael and Hibbert (*Ann.*, 1908, **364,** 64) have again proven the nitrile structure.

Hydrocyanic Acid, HCN (incorrectly written HCy), was discovered by Scheele in 1782, who made it from Prussian blue, hence the name prussic acid (German: Blausaeure). The Greek "kyanos," meaning "blue," and "gennao," "I make," form the roots of the word "cyanogen."

Hydrocyanic acid occurs in nature in several tropical plants, which have been studied by Greshoff (*Ber.*, 1890, **23,** 3537), Treub (*Ann. du jardin botanique de Buitenzorg*, **13,** 1—89, and *Rec. trav. chim.*, 1895, **14,** 276), Francis (*Anal.*, 1877, No. 13) and Bonenkamp and von Elk (*Pharm. Post*, **23,** 259) (see *Cyanogenetic Glucosides*, this vol., p. 101).

Hydrocyanic acid is formed by electric induction discharges through a mixture of cyanogen and hydrogen or a mixture of nitrogen and a volatile or gaseous hydrocarbon. Dewar (*Chem. News*, 1879, **39,** 282) formed it direct out of its elements in the electric arc. Chloroform and ammonia passed through a red hot tube form ammonium cyanide. Methylamine breaks down when passed through a red hot tube, forming hydrocyanic acid, ammonia, methane and hydrogen; conversely

when hydrocyanic acid is reduced methylamine is obtained. (Wuertz, *Ann. chim. phys.*, [*iii*], **30**, 454).

Many nitrogen-free organic compounds when oxidised with nitric acid form hydrocyanic acid. Kuhlmann (*Ann. Chem. Pharm.*, 1839, **29**, 284) states that by passing alcohol vapors and nitrogen oxide over spongy platinum, hydrocyanic acid is formed.

The kernels of *Pomeæ* (order *Rosaceæ*) and the fruits of *Amgydaleæ* form small quantities of hydrocyanic acid by maceration. Liebig and Woehler (*Ann. Chem. Pharm.*, 1837, **22**, 1) studied the splitting up of amygdalin. Amygdalin is decomposed in contact with water by emulsin, which is also present in bitter almonds.

$$\underset{\text{Amygdalin.}}{C_{20}H_{27}NO_{11}} + 2H_2O = HCN + \underset{\text{Benzaldehyde.}}{C_6H_5CHO} + \underset{\text{Glucose.}}{2C_6H_{12}O_6}$$

Theoretically, 100 parts of amygdalin should yield 5.91 of hydrocyanic acid, and the practical results are not far removed from this proportion. The following are the usual proportions of amygdalin contained in, and of hydrocyanic acid obtainable from, various natural sources:

	Amygdalin contained.	Yield of HCN.
Bitter-almond pulp,	4.25%	0.25 %
Cherry-stone kernels,	3 %	0.17 %
Wild service kernels,	1.5 %	0.08 %
Wild service flower, root and bark,	1 %	0.06 %
Sweet cassava root,	0.3 %	0.017%
Bitter cassava root,	0.45%	0.027%

To the presence of hydrocyanic acid the poisonous properties of the essence of cherry kernels, bitter-almond water (and the crude oil), and laurel water are due. Good Kirchwasser sometimes contains upward of 1 grain of hydrocyanic acid to the pint, and inferior specimens often four times as much.

The following are the usual proportions of real hydrocyanic acid present in certain preparations containing it:

Crude bitter-almond oil,	8 to 15%
Bitter-almond water,	0.25 to 1%
Cherry-laurel oil,	2 to 3%
Cherry-laurel water,	0.25 to 0.75%
Cluster cherry oil,	9 to 10%

Finally, hydrocyanic acid is formed by the destructive distillation of nitrogen containing organic compounds of vegetable and animal origin. Le Bon and Noel (*Compt. Rend.*, 1880, **90**, 1538), as well as Haber-

mann (*Zt. f. physiol. Chem.*, **37,** 1) studied the quantities of hydro-cyanic acid in tobacco smoke. Of large commercial importance is the hydrocyanic acid formed in the manufacture of illuminating gas.

Wade and Panting (*Trans.*, 1898, **73,** 255) prepare hydrocynic acid by dropping a mixture of equal volumes of concentrated sulphuric acid and water on pieces of potassium cyanide (98%). The evolved hydrogen cyanide is practically free from moisture and a nearly theoretical yield is obtained.

The method generally used for the preparation of hydrocyanic acid is by the distillation of potassium ferrocyanide and dilute sulphuric acid:

$$2K_4Fe(CN)_6 + 3H_2SO_4 = 6HCN + K_2Fe_2(CN)_6 + 3K_2SO_4.$$

Trautwein suggests 15 parts potassium ferrocyanide, 9 parts sulphuric acid and 9 parts water, while Woehler recommends 10 parts potassium ferrocyanide, 7 parts sulphuric acid and 14 parts water. The evolved vapours should be first passed over potassium cyanide to take out any sulphuric acid which might be carried over and then passed either over calcium chloride or through a solution of calcium chloride; in the latter case the dehydrated hydrocyanic acid swims on the top of the calcium chloride solution.

Dilute hydrocyanic acid of the strength required for medicinal use may be conveniently prepared by decomposing silver cyanide with an equivalent amount of dilute hydrochloric acid. 5 c.c. hydrochloric acid (sp. gr. 1.163) should be mixed with 5 c.c. of distilled water, and the liquid shaken in a stoppered bottle with 6 grm. of silver cyanide. The precipitate is allowed to subside and the clear liquid poured off. The hydrocyanic acid of the French Codex and the United States Pharmacopœia is prepared in this way. Another very convenient plan is to decompose the double cyanide of potassium and zinc (a very stable salt) with tartaric acid. 22 grains of the double cyanide, $K_2Zn(CN)_4$, and 49 grains of tartaric acid dissolved in an ounce of water, will yield, on filtration or decantation from the precipitate, a liquid containing 2% HCN. Or 9 grm. of tartaric acid may be dissolved in 93 c.c. of water, transferred to a stoppered flask and 4 grm. potassium cyanide added. The liquid is then well agitated and decanted when the precipitate has subsided.

Any of these processes will give a product containing 2% of real hydrocyanic acid. This is the strength of the acid of the Pharmaco-

pœias of Great Britain, United States, Germany, Norway and Switzerland. The acid of the Norwegian Pharmacopœia receives an addition of 0.1 % of strong sulphuric acid, as a preservative. The hydrocyanic acid of the London Pharmacopœia was of the same strength, but the preparations of the Edinburgh and Dublin Pharmacopœias contained 3.3 % of real acid. Scheele's acid is said to have a strength of about 5 %. The strength of the hydrocyanic acid met with in commerce varies to a serious extent.

Hydrocyanic acid, when dry, is a clear colourless liquid, has an odour similar to bitter almonds and produces a characteristic sensation in the throat. It is extremely poisonous; even small doses are fatal in a few seconds. Gattermann (*Ann.*, 1907, **357**, 319) describes an exceptionally sensitive taste reaction, detecting the smallest quantity of hydrocyanic acid, not detectable by the nose. If one smokes a cigar a characteristic taste occurs, if only a trace of hydrocyanic acid is present in the air.

Hydrocyanic acid boils at 26.5° and solidifies at −15°, forming a fibrous crystalline mass. Its sp. gr. determined by Gay-Lussac is 0.70583 at 7° and 0.6969 at 18°. Hydrocyanic acid is soluble in all proportions in water, alcohol and ether. In mixing it with water a lowering of temperature and a decrease in volume is noticeable.

When pure, hydrocyanic acid is very stable. The vapours passed through a hot porcelain tube decompose into hydrogen, cyanogen and nitrogen. The vapours burn in the air, with violet-blue flame, forming carbon monoxide, water and nitrogen. Hydrocyanic acid is one of the weakest acids and only temporarily reddens litmus paper, its aqueous solution having a low electric conductivity. Heated with metallic potassium or sodium it forms the corresponding cyanides. It reacts with certain metallic oxides either in solution or suspension. It only reacts with carbonates in the presence of a base forming double cyanide, for instance:

$$ZnO + K_2CO_3 + 4HCN = Zn(CN)_2.2KCN + 2H_2O + CO_2.$$
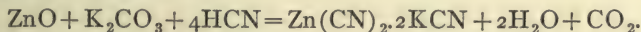
Both phenol and boracic acid will partly decompose potassium cyanide, forming hydrocyanic acid.

If hydrocyanic acid is passed into a solution of hydrogen peroxide, oxamide is formed. Aqueous solutions of hydrocyanic acid are not very stable, a brown residue being formed, beside splitting up into formic acid and ammonia:

$$HCN + 2H_2O = NH_3 + HCOOH.$$
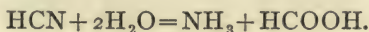
By the addition of small quantities of mineral acids the aqueous solution is much more stable, yet the decomposition into ammonia and formic acid eventually takes place.

Bitter-almond Water and Cherry-laurel Water contain ammonium cyanide and benzaldehyde cyanohydrin in addition to free hydrocyanic acid. The gravimetric method of Feldhaus (*Zt. f. Anal. Chem.*, 1864, **3**, 34) is usually used to determine the cyanogen present. 100 c.c. of the water are placed in a stoppered bottle to which is added about 1 grm. silver nitrate in 10 c.c. water, 2 to 3 c.c. of strong ammonia (sp. gr. 0.96) making the solution strongly alkaline, when it is *at once* acidified with dilute nitric acid, carefully avoiding a large excess. The process must be performed in the cold and with the greatest possible rapidity. The precipitated silver cyanide is filtered, dried, ignited and weighed as described on page 457.

According to the German Pharmacopœia IV, the cyanogen contents are estimated as follows: 25 c.c. bitter-almond water are diluted with 100 c.c. water, to which is added 1 c.c. potassium hydroxide and a trace of sodium chloride and then while stirring a $N/10$ silver nitrate solution is allowed to flow in until a permanent turbidity is obtained; a minimum of 4.5 c.c. and a maximum of 4.8 c.c. must be required. The United States Pharmacopœia recommends the following method for bitter-almond oil: Mix in a 100 c.c. flask 1 grm. of the oil of bitter-almond to be tested, with sufficient water and freshly precipitated magnesium hydroxide (free from chlorides) to make an opaque mixture of about 50 c.c. Add to this 2 or 3 drops of potassium chromate and then from a burette add $N/10$ silver nitrate solution until a red tint is produced which does not again disappear by shaking; not less than 7.5 c.c. nor more than 14.9 c.c. $N/10$ silver nitrate should be required, each c.c. corresponding to 0.002684 grm. HCN. By this method all the free acid is neutralised by the addition of magnesia, so that potassium dichromate may be used instead of the neutral salt.

Toxicology of Hydrocyanic Acid.—Hydrocyanic acid, in any form, and however administered, is an intensely active poison to every animal, with the exception of the frog. Broadbent (*Pharm. J.*, 1891, [iii], **21**, 136) claims that the frog becomes gradually narcotised by the inhalation of hydrocyanic gas, but recovers when brought into the open air. When swallowed, injected, inhaled and sometimes even when merely applied to the sound skin, and when applied to the mucous membrane of the eye, its action is energetic. An internal dose of three

to four grains of real hydrocyanic acid is generally fatal to the human subject, and less than one grain has been known to cause death.

Chlorine and ammonia appear to act as antidotes in cases of hydrocyanic acid poisoning, but their mode of action is obscure, since the presumed products of their action—namely, cyanogen chloride and ammonium cyanide, are as poisonous as hydrocyanic acid itself.

The detection of hydrocyanic acid in the body is rendered difficult by the great facility with which the acid decomposes. Calvi and Malacarne (*Giorn. Farm. Chim.*, 1906, **56**, 5) made some experiments regarding the stability of hydrocyanic acid in corpses. They were able to detect hydrocyanic acid in the intestines 22 days after death had taken place. After 30 days only traces could be found. The decomposition is accelerated by the action of the air and organic substances, and is retarded in the presence of 95% alcohol, therefore the parts of a corpse which are to be examined should be conserved with alcohol. Reichardt mentions a case in which he succeeded in detecting hydrocyanic acid two months after death. He obtained no definite results from the urine in this case, but the distillate from the organs previously acidified with tartaric acid yielded affirmative results both by the guaiacum and the Prussian blue tests.

On opening the stomach and intestines the odour of hydrocyanic acid is often perceptible. These viscera are often quite natural in appearance, but sometimes more or less inflamed and congested. The lungs, liver, spleen and kidneys are always found gorged with blood. The venous system is invariably gorged with blood, the arteries being empty. The blood has undergone change; it may be black or oily, or of a cochineal-red colour. It often smells of the poison, which may frequently be distilled from it.

To detect hydrocyanic acid in the contents of a stomach the analyst should proceed as follows:

Note the reaction of the liquid portion. If not distinctly alkaline to litmus, the poison (if present) was probably administered as free hydrocyanic acid, and not as cyanide of potassium. (The various double cyanides used for electro-deposition of metals have a neutral reaction in the absence of excess of potassium cyanide).

Stir up the stomach and its contents with cold water, and introduce the thick liquid into a flask adapted to a Liebig's condenser, allowing the end of the condenser tube to be immersed in a small quantity of water. Apply a moderate heat to the flask (best by an external bath

of salt water), and distil over about half the liquid. To the distillate apply carefully the silver nitrate, Prussian blue and thiocyanate tests, as described on page 453. It is preferable to avoid any addition of acid to the liquid to be examined, as the saliva contains traces of thiocyanates, which might possibly yield traces of hydrocyanic acid on distillation with mineral acid. If the distillate has given negative results when tested for hydrocyanic acid, continue the distillation after rendering the contents of the flask distinctly acid with tartaric acid. If hydrocyanic acid be now found in the distillate, the poison must have been present as a readily decomposable cyanide. The guaiacum-copper reaction (page 480) is recommended as a preliminary test. If the distillate gives no indication of hydrocyanic acid by this test, it is considered useless to ·proceed further. But if an affirmative result is obtained, other tests should be employed. If only a small quantity of hydrocyanic acid be present in the distillate, it is most conveniently detected by precipitating it with silver nitrate, and applying the iron and sulphur tests to the precipitate (see page 479).

Before finally concluding that all metallic cyanides are absent, it is desirable to repeat the distillation after adding a considerable excess of moderately dilute sulphuric and hydrochloric acids. Ferrocyanides, ferricyanides and mercuric cyanide will in this case be decomposed. Hence the absence of ready formed ferrocyanides or ferricyanides should be previously ascertained by testing portions of the acidified contents of the stomach with solutions of ferric and ferrous salts.

Beckurts (*Chem. News*, 1883, **48**, 199) and Taylor (*loc. cit.*, 1884, **50**, 227) have pointed out that potassium ferrocyanide is less stable in presence of dilute acids than is generally supposed. Carbon dioxide at 74° liberates hydrocyanic acid from it, with precipitation of potassium ferrous ferrocyanide, $K_2FeFe(CN)_6$, and casein, peptone, and artificial digestive fluid excercise a similar action at 40°. Autenrieth (*Arch. Pharm.*, 1893, **231**, 99) considers, therefore, that the only certain way to detect hydrocyanic acid or simple cyanides in presence of ferrocyanides is by Jacquemin's process. He distils the material with a considerable amount of sodium hydrogen carbonate, and examines the distillate for hydrocyanic acid. If the presence of mercuric cyanide be suspected, sulphuretted hydrogen water must be also added, as the sodium hydrogen carbonate does not itself decompose mercuric cyanide. Or mercuric cyanide may be decomposed by adding a few bright strips of zinc to the liquid before commencing the distillation. Ferricy-

anides, thiocyanates, sulphates and ammonium salts do not interfere with the process.

Mercuric cyanide may be decomposed by distillation with hydrochloric acid. After testing for other simple cyanides, mercuric cyanide can be estimated by acidifying the mixture, after distillation with sodium hydrogen carbonate, with tartaric acid, adding ammonium chloride in excess, and again distilling. A double chloride of mercury and ammonium is said to be formed, and hydrocyanic acid distils with the steam. To test for cyanides in presence of ferrocyanides, Lopes heats the substance to 100° with milk of lime, in order to decompose ammonium salts, which, if present, may react with ferrocyanide to form volatile ammonium cyanide. When all the ammonia has been driven off, the solution is filtered and distilled with excess of sodium hydrogen carbonate as recommended by Autenreith.

G. Portmann (*J. Soc. Chem. Ind.*, 1886, **5**, 679) gives the following test for the detection of minute quantities of hydrocyanic acid. It is based on the formation of a nitroprusside by the action of a metallic nitrite on potassium cyanide in presence of a ferrous salt. The liquid to be tested is treated with a few drops of a solution of potassium nitrite, two or three drops of ferrous sulphate solution, and enough dilute sulphuric acid added to cause the yellow-brown colour of the basic ferric salt to become a light yellow. The liquid is then boiled, cooled, the excess of iron precipitated with ammonia, and the solution filtered. A few drops of freshly prepared ammonium sulphide are added, when, if any hydrocyanic acid were present in the original liquid, a violet colouration immediately appears, which changes rapidly through blue and green to a yellow colour. Very minute traces of hydrocyanic acid produce a bluish-green colour, rapidly changing to greenish-yellow. Portmann estimates the delicacy of this test at 0.00003 grm. HCN dissolved in 10 c.c. of water. That is, 1:312,000.

One of the main causes of the disappearance of hydrocyanic acid in the dead body is its reaction with ammonium sulphide, and probably with other sulphuretted substances produced by putrefaction, whereby thiocyanates are formed. Therefore, where hydrocyanic acid is to be sought for a considerable time after death, it may sometimes be detected by rendering the materials distinctly, but not excessively, alkaline with potassium hydroxide and then adding excess of alcohol. The liquid is filtered and evaporated to dryness. The residue is redissolved in water, acidified with hydrochloric acid, and ferric chloride

added, when a red colour will be produced if a thiocyanate were present in the stomach.

In applying this test it must not be overlooked that thiocyanates are normally present in the saliva. Hence they exist in traces in the stomach and can be detected therein by ferric chloride.

A method for the estimation of hydrocyanic acid in vegetable and animal tissues by Waller (*Proc. Roy. Soc.*, 1910, [B], **82,** 574) consists in distilling the tissues to be examined with dilute acid, receiving the distillate in the "picrate fluid," which is then placed for an hour in an incubator at 40°, when the tint of same is compared with a standard. The "picrate fluid" employed is a solution containing 0.05% picric acid and 0.5% sodium carbonate. The errors of manipulation are claimed to be very small on minute quantities of hydrocyanic acid, and it was found that hydrocyanic acid goes to the heart and brain rather than to the muscles. This method was used to prove that the formation of hydrocyanic acid from laurel leaves takes place subsequent to death.[1]

Antidotes for Cyanide Poisoning.—Geppert (*Zt. f. klin. Med.* [*Berlin*], 1889, **12,** 208) states that in hydrocyanic acid poisoning less oxygen is used and therefore less carbon dioxide formed than under normal conditions. Owing to the presence of hydrocyanic acid the tissues lose their property of combining with oxygen and the poisoning is an internal asphyxiation of the organs, in the presence of an excess of oxygen. Therefore, the administering of oxygen gas and subcutaneous injections of hydrogen peroxide are recommended. Internal application of 2 to 3% hydrogen peroxide is given together with artificial respiration.

Other well-known antidotes consist in breathing ammonia or chlorine from moist chloride of lime; and ferrous sulphate followed by potassium carbonate, emetics and warmth. Kobert recommends 0.001 grm. (1/60 grain) atropine hypodermically.

The Mining Regulations Commission of Transvaal (*Eng. Min. Jour.*, 1910, **90,** 1047 and 1092) recommend the use of ferrous sulphate, followed immediately by potassium hydroxide.

Simple metallic cyanides are formed by replacing the hydrogen atom in hydrocyanic acid by a metal; the latter is, therefore, bound to a carbon atom. Silver cyanide forms an exception to this. A spontaneous molecular change often takes place, inasmuch as iso-

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[1] Chapman (analyst, 1909, **34,** 475 and 1910, **35,** 469) has shown that the picrate reaction is not a specific indication of hydrogen cyanide but is given by other substances. See also Lauder and Walden, *Analyst*, 1911, **36,** 266.

nitrile is frequently formed when reacting with alkyl iodides, instead of alkyl cyanides, as would be expected.

Metallic cyanides do not occur in nature, but occasionally form as by-products in technical processes; in the Le Blanc soda melt sodium ferrocyanide is found, in blast furnace slags titanium cyanogen nitrogen is found, etc.

The alkali, alkali earth and mercury cyanides are soluble in water, while the cyanides of other metals are insoluble; however, the latter are easily soluble in alkali cyanide solutions forming double cyanides.

The alkali cyanides, by exclusion of air, can be molten without decomposition, all the others decomposing at red heat. The cyanides of mercury, silver and gold give off cyanogen when heated. The simple metal cyanides set free all of the cyanogen as hydrocyanic acid when distilled with hydrochloric acid. Heated with concentrated sulphuric acid the corresponding metal sulphates, ammonium sulphate and carbon monoxide are formed.

Alkali and alkali earth cyanides heated to red heat with powdered magnesium form the metal carbides and magnesium nitride.

Copper, cadmium, cobalt, nickel and zinc cyanides react with magnesium powder, forming the corresponding metal, charcoal and magnesium nitride, accompanied with an intense glowing and a weak detonation; mercury and silver cyanides react the same, only the reaction takes place at a much lower temperature. All cyanides detonate when heated with potassium nitrate and chlorate.

As stated on page 465, aqueous solutions of hydrocyanic acid have a low electric conductivity, therefore alkali and earth alkali cyanides, in solution, are strongly hydrolysed, and in the case of alkali cyanides, the solutions behave as if they contained alkali hydroxide and hydrocyanic acid. In fact, a solution of an alkali cyanide smells distinctly of hydrocyanic acid.

The simple metal cyanides are typified by the alkali (sodium and potassium) cyanides. Their ever growing industrial application has led to the invention of processes for their economic production and also made valuable the by-products of the gas and beet-sugar industry.

The U. S. Bureau of Census (Bulletin No. 92, 1905) states that nearly 13,000,000 lbs. of potassium cyanide were manufactured and imported into the United States.

Outside of the application of alkali cyanide in the metallurgy of gold, it is used by electroplaters, photographers, etc., as a means of case

hardening steel, for agricultural purposes as an insecticide for fruit trees by fumigation and an insecticide of the soil by injections (Mamelle, *Compt. rend.*, 1910, **150**, 50).

Ammonium Cyanide, NH_4CN or NH_4Cy. The "Cy" symbol is often used to denote the radical " $-CN$."

Ammonium cyanide occurs in crude illuminating gas. It can be formed by passing ammonia over red hot charcoal (Langlois, *Jahresbericht*, 1877, **22**, 84). Figuier (*J. Phar. Chim.*, [*vi*], **13**, 314) formed ammonium cyanide by allowing an electric current to pass through a mixture of methane and nitrogen in the dark. The preparation from chloroform and ammonia has already been alluded to under hydrocyanic acid. The best method of preparing ammonium cyanide is by heating a mixture of potassium ferrocyanide with 2 parts of ammonium chloride (Bineau, *Ann. Chim. Phys.*, **67**, 231).

$$K_4Fe(CN)_6 + 4NH_4Cl = 4NH_4CN + 4KCl + Fe(CN)_2.$$
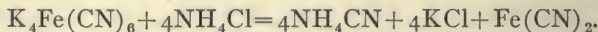
The vapors are condensed in a receptacle which is cooled with ice and salt.

Ammonium cyanide crystallises in colourless cubes, boils at 36°, and has according to Bineau (*Ann. Chim. Pharm.*, 1839, **32**, 230) a vapor density of 0.79. It reacts alkaline, smells of ammonia and hydrocyanic acid and is very poisonous. It is easily soluble in water and alcohol. At ordinary temperatures it decomposes into ammonia and azulminic acid, the dcomposition being accelerated by higher temperatures. Ammonium cyanide vapours burn in the air.

Sodium Cyanide, $NaCN$, can be prepared by Johannis's method (*Ann. Chim. Phys.*, 1882, [*v*], **26**, 484) by passing dry hydrocyanic acid gas into alcoholic sodium hydroxide. Rogers (*Phil. Mag. J.*, 1875, **4**, 93) prepares it by precipitating the mercury out of a mercury cyanide solution, with a quantitative amount of sodium sulphide, or by treating a barium cyanide solution with sodium sulphate, and evaporating the filtrate *in vacuo.*

Sodium cyanide crystallises from water or boiling alcohol (75%) with two molecules of water of crystallisation. If cold alcohol is used it crystallises with one molecule of water of crystallisation.

Potassium cyanide, KCN, is formed by passing nitrogen over a mixture of charcoal and potassium carbonate at a high temperature, also by melting nitrogen containing organic compounds with potassium carbonate, for example, animal offal together with potassium carbonate.

The best known method for preparing potassium cyanide is by heating potassium ferrocyanide in an iron crucible in the absence of air:

$$K_4Fe(CN)_6 = 4KCN + FeC_2 + N_2.$$
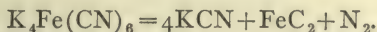
Liebig (*Ann. Chem. Pharm.*, 1842, **41,** 285) suggests heating a mixture consisting of 8 parts of potassium ferrocyanide and 3 parts potassium carbonate:

$$K_4Fe(CN)_6 + K_2CO_3 = 5KCN + KCNO + Fe + CO_2.$$
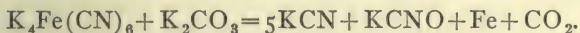
If sodium cyanide may be contained in the potassium cyanide in small amount, then Erlenmeyer's method (*Ber.*, 1876, **9,** 1840) is very handy. He melts potassium ferrocyanide together with metallic sodium:

$$K_4Fe(CN)_6 + 2Na = 4KCN + 2NaCN + Fe.$$
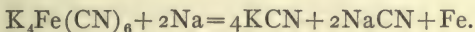
The melt is powdered and treated with boiling 50% alcohol, from which the potassium cyanide precipitates on cooling, as a fine crystalline powder.

Absolutely pure potassium cyanide can be prepared according to Wiggers (*Zt. f. anal. Chem.*, 1876, **15,** 448) by passing pure dry hydrogen cyanide into an alcoholic potassium hydroxide solution, and the potassium cyanide formed will fall out as a crystalline powder. The powder is washed on the filter with alcohol and dried over sulphuric acid.

Potassium cyanide crystallises out of water in colourless octahedrons with water of crystallisation. Out of a melt it crystallises in cubes. It is very soluble in water; dissolves in 2 parts of water at 25° and in one part of boiling water; it is soluble in about 80 parts of alcohol, the solubility considerably increasing by diluting the alcohol with water; it decomposes slowly in water to potassium carbonate, potassium formate and ammonia. It deliquesces in moist air, giving off an odour of hydrocyanic acid. It melts at dull red heat, volatilising at a white heat without decomposition.

Potassium cyanide reacts strongly alkaline and is poisonous, even in small quantities, although not so poisonous as hydrocyanic acid. The antidotes, besides those mentioned on page 470, are freshly precipitated ferric hydroxide with sodium carbonate, cobalt nitrate and 10 grm. iron sulphate with 1 dram tincture of iron in 1 oz. water.

Potassium cyanide is an excellent reducing agent at higher temperatures for metal oxides, and according to Eiloart (*Chem. News*, 1886, **54,** 88) at a light red heat it reduces carbon dioxide to carbon monoxide, forming cyanate. The aqueous solution of potassium cyanide dissolves many metals (as iron and zinc), evolving hydrogen; but the noble metals, gold and silver, will only go into solution by passing air through the potassium cyanide solution.

Sodium and potassium cyanides, both being commercial articles, have similar modes of manufacture, and are met with as much in mixtures as in the simple product; therefore, a résumé of the commercial and economic methods of manufacture is here given.

The old method of introducing offal, leather scrap, old shoes, horn pieces, hoofs and claws, wool waste, sinews, hair and other tankage waste, etc., into a potash melt is only very rarely met with now, and is possibly more of historical than commercial interest. The cyanide formed by this process is first obtained as potassium ferrocyanide, containing sulphur and other cyanogen compounds as impurities. The potassium ferrocyanide is separated from the impurities by crystallisation and the so-formed potassium ferrocyanide is either heated alone with potash or with metallic sodium as already described (see page 473). On account of the increasing demands for cyanide in industrial pursuits, many inventions have appeared to manufacture either sodium or potassium cyanide very cheaply. A very large number of processes have been suggested to make cyanides or hydrocyanic acid synthetically, yet none of these methods have been able to claim a long industrial life.

In the coal-gas industry the cyanogen compounds are absorbed in the purification mass, which is known, after being pressed, as blue cake or sludge, and is worked into commercial cyanogen compounds.

The mass contains Prussian blue, sulpho-cyanogen compounds, ammonia compounds, etc. After gaining the ammonia, the cyanogen compounds are worked into ferrocyanide salts of sodium or potassium and then converted into sodium or potassium cyanide as already described.

Bueb (*J. Gas Lighting*, 1900) passes the gases from the destructive distillation of bagasse over glowing pieces of earthenware, thus converting the organic amines (trimethylamine, pyridine, etc.) into hydrocyanic acid, which is absorbed by potassium or sodium hydroxide.

Pfleger (*J.*, 1896) passes a current of ammonia gas over a heated mixture of potassium carbonate and charcoal. Pfleger (United States Patent, 686, 949, 1901) passes ammonia into molten sodium to which charcoal has been added.

The oxidation of alkali thiocyanate with nitric acid and the absorption of the formed hydrocyanic acid by alkali hydroxide is also used.

Sodium cyanide, potassium cyanide and mixtures of sodium and potassium cyanide form the merchantable products generally found in commerce. In the gold cyaniding plants, where cyanide is used in large quantities, sodium cyanide is mostly used; sodium cyanide carrying more cyanogen, on account of the lower molecular weight of sodium than potassium cyanide thus saving storage and freight charges, the advantage of less handling and possibly lower cost, sodium compounds being generally cheaper than the corresponding potassium compounds.

It is customary in commerce to mark cyanides, whether it is potassium cyanide, sodium cyanide or mixtures of both as their equivalent in potassium cyanide; thus one finds products testing over 100% KCN. This at once shows that the product is either sodium cyanide or a mixture of sodium and potassium cyanides. The highest technical cyanide met with at present is what is known as 130% cyanide, which means that it is equivalent to 130% potassium cyanide. A product of this nature represents a sodium cyanide of 98% purity.

Calcium Cyanide, $Ca(CN)_2$, can be prepared by saturating calcium hydroxide with hydrocyanic acid.

Calcium cyanide crystallises in cubes. Aqueous solutions of calcium cyanide easily decompose, especially in the presence of free hydrocyanic acid. Evaporated *in vacuo* over sulphuric acid, small crystals are formed, having the formula $Ca(CN)_2.3CaO + 15H_2O$. If these crystals are dried *in vacuo*, they completely decompose into hydrocyanic acid and calcium hydroxide (Johannis, *Ann. chim., phys.*, 1882, [v], **26,** 496).

Strontium Cyanide, $Sr(CN)_2 + 4H_2O$, is prepared similarly to calcium cyanide and shows similar properties. It crystallises in orthorhombic prisms.

Barium Cyanide, $Ba(CN)_2$, according to Marguerite and Sourdeval (*J.*, 1860, 224) is prepared by passing air over a mixture of barytes or barium carbonate and charcoal at a high temperature. Hydrated barium cyanide, $Ba(CN)_2 + 2H_2O$, is prepared by allowing dry hydrocyanic acid to react with crystallised barium hydroxide and evaporating

the obtained solution *in vacuo* over sulphuric acid and potassium hydroxide (Johannis, *Ann. chim. phys.*, 1882, [v], **26**, 489).

Barium cyanide is the most stable of the alkali earth cyanides. The hydrated barium cyanide crystals lose one molecule of water by being dried *in vacuo* over sulphuric acid. The second molecule can be driven off by drying in a current of air at 75 to 100°.

10 parts water dissolve 8 parts barium cyanide at 14°, and 10 parts alcohol (70%) dissolve 1.4 parts at 14°.

Zinc Cyanide, $Zn(CN)_2$, is easily formed by heating organic nitrogenous matter with zinc dust. Hydrocyanic acid passed over zinc oxide, whether it has been previously glowed or not, converts it into zinc cyanide. Woehler's method (*Berzelius's Jahresber.*, 1841, **20**, 152) of precipitating zinc cyanide by passing hydrocyanic acid into a zinc acetate solution is the most convenient for its preparation.

Zinc cyanide is a snow-white, tasteless powder, insoluble in water and alcohol. Decomposes only at high temperatures.

Alkali hydroxides dissolve zinc cyanide, forming according to Sharwood (*Eng. and Min. J.*, 1904, **77**, 845) a double salt of alkali zinc cyanide:

$$4KOH + 2Zn(CN)_2 = K_2Zn(CN)_4 + K_2ZnO_2 + 2H_2O.$$
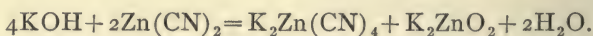
Cuprous Cyanide, $Cu_2(CN)_2$, is prepared either by precipitation with potassium cyanide from a copper sulphate solution in the presence of sulphurous acid, or by precipitating from a cuprous chloride solution with an alkali cyanide. Cuprous cyanide is colourless, insoluble in water and dilute mineral acids; however, potassium cyanide and ammonia dissolve it, forming a double salt, potassium cuprous cyanide, from which hydrogen sulphide will not precipitate copper sulphide, but moreover, freshly precipitated copper sulphide is dissolved in a solution of potassium cyanide.

Silver Cyanide, AgCN, is prepared by precipitation from a silver nitrate solution with hydrocyanic acid or alkali cyanide. It forms a white cheesy precipitate, differing from silver chloride in so far that it is not effected by light. Silver cyanide is insoluble in water and dilute acids, but is readily soluble in alkali cyanide and ammonia. Concentrated hydrochloric acid decomposes it into silver chloride and hydrocyanic acid and hydrogen sulphide gives silver sulphide and hydrocyanic acid with the silver cyanide. Silver cyanide when ignited decomposes into silver, cyanogen and paracyanogen.

Gold Cyanide exists as aurous cyanide, AuCN, and auric cyanide $Au(CN)_3$. Aurous cyanide is formed by heating potassium aurous cyanide with hydrochloric acid. It forms a light yellow crystalline powder, which is insoluble in water and mineral acids. It is, however, easily soluble in potassium cyanide and ammonia. When aurous cyanide is ignited it forms metallic gold and cyanogen.

Mercuric Cyanide, $Hg(CN)_2$, is prepared by dissolving mercuric oxide in an excess of hydrocyanic acid. Mercuric cyanide crystallises in colourless quadratic prisms and is extremely poisonous.

Mercuric cyanide is almost the only simple cyanide of a heavy metal which is soluble in water. Owing to its stability it reacts in an anomalous manner. Thus it does not respond to the iron test for cyanides (page 478), and is not precipitated by silver nitrate. It yields, however, a yellowish-white precipitate of palladious cyanide on addition of palladious nitrate. Mercuric cyanide is not precipitated by alkalies, but by boiling with hydrochloric acid hydrocyanic acid is evolved and mercuric chloride formed. Solutions of mercuric cyanide are readily decomposed by hydrogen sulphide, and, after separation from the precipitated mercuric sulphide, the cyanide in the liquid can readily be estimated by Liebig's test (page 481). Mercuric cyanide may also be decomposed by digesting the solution with cadmium cyanide, $Cd(CN)_2$, in which the cyanide is readily estimated.

Owing to the tendency to form mercuric cyanide, many simple and double cyanides are decomposed by boiling with yellow mercuric oxide and water. This is true of ferrocyanide and ferricyanide of potassium and also of Prussian blue, but not of cobalticyanides.

No mercurous cyanide is known; on adding mercurous nitrate to a liquid containing hydrocyanic acid, or a metallic cyanide, metallic mercury separates, and soluble mercuric cyanide is formed. A similar reaction occurs on treating calomel with excess of hydrocyanic acid.

When dry mercuric cyanide is heated it decomposes, with formation of metallic mercury, paracyanogen and cyanogen gas.

A hot solution of mercuric cyanide readily dissolves yellow mercuric oxide. The resultant solution has been recommended instead of solid mercuric oxide for the separation of cobalt from nickel.

Detection of Metallic Cyanides.

These reactions apply to all soluble cyanides except mercuric cyanide above.

Dilute hydrochloric or sulphuric acids will evolve poisonous hydrocyanic acid, having an odour of bitter almonds. Soluble cyanides will react in the cold, while insoluble cyanides will only evolve hydrocyanic acid on warming.

All metallic cyanides are decomposed by heating with concentrated sulphuric acid, evolving carbon monoxide gas. This reaction also includes mercuric cyanide, which evolves carbon dioxide and sulphur dioxide, besides carbon monoxide.

An excess of silver nitrate solution added to an alkali cyanide solution forms a white cheese-like precipitate which closely resembles silver chloride, except that it does not darken in sunlight. This precipitate, silver cyanide, is soluble in an excess of the alkali cyanide solution, forming a complex cyanide—potassium silver cyanide:

$$AgCN + KCN = KAg(CN)_2.$$
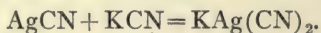
This complex salt is decomposed by adding more silver nitrate solution:

$$KAg(CN)_2 + AgNO_3 = KNO_3 + 2AgCN.$$
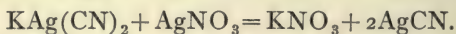
Differing from silver chloride, this silver cyanide precipitate evolves hydrocyanic acid when heated with hydrochloric acid. With it one can also detect cyanides in presence of bromides and iodides. Silver cyanide is insoluble in water and dilute nitric acid, but is soluble in hot concentrated nitric acid, hence silver cyanide can be separated from silver chloride, and the silver of the silver cyanide in solution can be precipitated as silver chloride by the addition of hydrochloric acid or a soluble chloride. If the solution of silver cyanide in hot nitric acid (sp. gr. 1.2) be filtered from the insoluble silver chloride and allowed to cool without agitation, the silver cyanide is deposited in a semi-gelatinous form. On gentle agitation it collects suddenly into opaque masses, which under the microscope appear as groups of needles. On ignition silver cyanide gives off cyanogen gas, leaving metallic silver and a brown product—paracyanogen. This latter product volatilises with difficulty, differing thus from silver chloride, which fuses without decomposition. Silver cyanide, when gently heated with ammonia, is deposited in needles, while silver chloride similarly treated yields octahedra.

On adding a solution of ferrous sulphate to an alkali cyanide solution, oxidising with a small quantity of hydrogen peroxide, sodium perborate, nitric acid or any other oxidising product, then acidifying

with hydrochloric acid, a Prussian blue precipitate (or bluish-green colouration) of ferric ferrocyanide is produced:

$$FeSO_4 + 6KCN = K_2SO_4 + K_4Fe(CN)_6$$
$$3K_4Fe(CN)_6 + 4FeCl_3 = 12KCl + Fe_4(FeC_6N_6)_3.$$
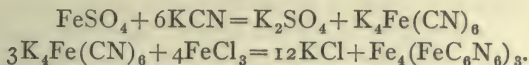
The cyanide solution must be alkaline, thus always some alkali hydroxide is added with the ferrous sulphate solution. In cases where only traces of cyanide are present, a bluish-green solution is formed, which precipitates flocculent Prussian blue after standing some time. This characteristic reaction is very sensitive and one part hydrocyanic acid in 50,000 parts of water can be indicated. The reaction is not applicable in presence of ferrocyanides or ferricyanides.

On evaporation to dryness at a steam heat, after addition of yellow ammonium sulphide, a thiocyanate (sulpho-cyanide) is formed. On treating the residue with water, filtering if necessary, acidifying with hydrochloric acid, to destroy any ammonium sulphide, and adding a few drops of ferric chloride solution, a blood-red colour is produced, due to the formation of ferric thiocyanate. The colour is distinguished from that due to an acetate or formate by being unaffected by dilute hydrochloric acid, and from that produced by a meconate by being readily destroyed on addition of mercuric chloride. If only a drop or two of the ferric chloride has been used, thus preventing an excess of iron solution, then on agitation of the liquid with ether the thiocyanate dissolves and colours the ethereal layer red. This test can be applied to silver cyanide. Free hydrocyanic acid or simple cyanides can be detected in the presence of a ferrocyanide, ferricyanide or thiocyanate by mixing the suspected liquid with tartaric acid, warming it, and allowing the vapours to act on a drop of ammonium sulphide contained in a porcelain dish or watch-glass, inverted over the vessel containing the sample to be tested. After some time the cover is removed, the drop of liquid evaporated to dryness, and the residue treated as described above. This elegant and delicate test, which is due to Liebig, is said to indicate the presence of one part hydrocyanic acid in 4,000,000 parts of water (Link and Maeckel, *Zt. f. anal. Chem.*, 1878, **17**, 455).

Free hydrocyanic acid responds readily to all the above tests, but it is often advantageous to neutralise with a little weak solution of alkali hydroxide before testing; this is especially so in the thiocyanate test, but an excess of alkali hydroxide should be carefully avoided.

A useful test for free hydrocyanic acid is the blue colouration it produces with gum guaiacum in presence of compounds of copper. The test is commonly applied by moistening a slip of filter paper with a very dilute solution of copper sulphate, then with a tincture of guaiacum, and drying it. If the paper so prepared be immersed in a liquid containing a trace of hydrocyanic acid it acquires a bright blue colour. Hilger and Tamba (*J. Chem. Soc.*, Abstr., 1891, **60,** 1555) consider guaiacum test paper untrustworthy, and recommend that the suspected liquid should be treated in a porcelain dish with a drop of tincture of guaiacum, followed by a drop of copper sulphate solution.

A solution of an alkali cyanide will reduce mercurous nitrate, precipitating metallic mercury, thus differing from a halide. [1]

Estimation of Cyanides.

Hydrocyanic acid and the alkali and alkali earth cyanides can be estimated both volumetrically and gravimetrically, the volumetric estimation being the method generally used in industrial tests.

The behaviour of alkali cyanides toward indicators of neutrality shows that they can be estimated by alkalimetric methods. Litmus, phenol-phthalein or methyl orange, when used as an indicator by titration with standard hydrochloric acid, will titrate all the alkali cyanide present, just as if it was an alkali hydroxide. However, this method is not used in industrial tests, because alkali cyanide, which has been exposed to a moist atmosphere, will always contain alkali hydroxide and carbonate.

Various methods of estimating cyanides have been devised, but the best for free hydrocyanic acid as well as for alkali and alkali earth cyanides is the quantitative formation of silver cyanide by the addition of silver nitrate solution.

A convenient and fairly accurate method of assaying hydrocyanic acid and preparations containing it is to make a milk by grinding magnesia with water, and add sufficient of the mixture to a known measure of the liquid to be tested to render it opaque after agitation. Two drops of a saturated aqueous solution of neutral potassium chromate are then added, and the liquid titrated with $N/10$ solution of silver nitrate until a pale red tint is observed which does not disappear on agitation. This reaction corresponds to the formation of red silver chromate, which does not occur until the whole of the cyanide present

[1] For. detection of minute traces of hydrogen cyanide especially Lauder and Walden, *Analyst*, 1911, **36,** 266.

has been converted into silver cyanide.   1 c.c. of $N/10$ silver nitrate solution corresponds to 0.002702 grm. HCN.

The foregoing process is practically identical with that commonly employed for the estimation of chlorides.   Hence it follows that chlorides must be absent, or must be separately estimated and allowed for. A combination of the foregoing process with that next to be described would allow for an estimation of chlorides in presence of cyanides.

Liebig's Volumetric Method for the estimation of simple cyanides is the one mostly applied in commerce on account of its simplicity and accuracy.   Liebig's method is based on the fact that silver cyanide forms a soluble double salt with an aqueous solution of potassium cyanide:

$$2KCN + AgNO_3 = KAg(CN)_2 + KNO_3.$$
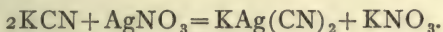
By the addition of an excess of silver nitrate the double salt potassium silver cyanide decomposes and silver cyanide is precipitated; a drop of the silver solution in excess causes a permanent turbidity of the solution.   This method only indicates the metallic cyanide present, free hydrocyanic acid giving an immediate precipitate with the silver solution.   Hence if free hydrocyanic acid is present, the solution must first be treated with a full equivalent of alkali hydroxide, but any great excess should be avoided as the results come too high.   However, the solution at the termination of the titration should be distinctly alkaline to litmus, otherwise more sodium hydroxide must be added (which will cause the disappearance of the turbidity), and then the titration continued till a permanent turbidity results.   If any alkali sulphides are present they must be separated before the titration starts, as the slightest amount of sulphides is detected in titrating by the formation of a yellow to brown colour in the solution.   The sulphides are precipitated from the alkali cyanide solution by the addition of freshly precipitated bismuth hydroxide (formed by the treatment of bismuth subnitrate with alkali hydroxide, filtering and washing the bismuth hydroxide out with water) or by the addition of freshly precipitated, lead carbonate, the insoluble sulphides being filtered off and washed. The presence of free ammonia retards the end point of the titration, giving thus erroneous results.   If free ammonia is present it should be neutralised with carbonic water to form ammonium carbonate, which has no effect on the end point of the titration.

To estimate the cyanogen in alkali cyanides, 2 grm. are weighed out

washed into a 250 c.c. volumetric flask, which is filled to the mark and thoroughly shaken.   25 c.c. of this solution is taken and placed in a beaker resting on a black surface.   Now a $N/10$ solution of silver nitrate is run in from a burette, with continual agitation, until a slight permanent turbidity results.   The number of c.c. $N/10$ silver nitrate multiplied by 2.601 = % CN, or multiplied by 2.702 = % HCN, or by 6.511 = % KCN.   Commercial metallic cyanide tests are generally calculated on the basis of potassium cyanide, therefore sodium cyanide would show a test of more than 100%. There is a tendency to give the tests showing the %CN present which is more scientifically correct.

FIG. 29.

Precautions must be taken to avoid inhaling the vapour or the fatal consequences of sucking some of the liquid into the mouth while pipetting the solution from the volumetric flask; to prevent any such occurrence the volumetric flask is supplied with a rubber stopper, through which the pipette and a bent glass tube fits; the pipette dips below the level of the liquid, while the bent glass tube comes just below the rubber stopper.   The bent tube is connected with a small rubber bulb, which on pressing exerts a pressure on the liquid, causing it to rise in the pipette (see Fig. 29).
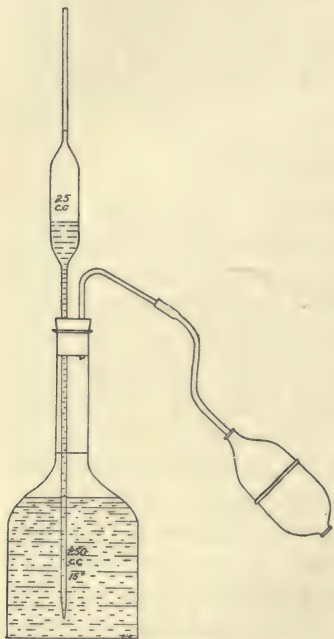
In a liquid containing alkali cyanide as well as free hydrocyanic acid, the amount of each of these may be ascertained by titrating first without addition of alkali hydroxide, and then continuing the process after adding it.   The volume of the silver solution first used represents the metallic cyanide, the second quantity the free hydrocyanic acid.

The addition of a few drops of potassium iodide to the solution before titrating by the Liebig's method is often recommended, when the permanent turbidity formed at the end of the reaction is of a yellowish hue instead of a bluish-white.   This is of no advantage

except in cases where an excess of alkali hydroxide or free ammonia are likely to be present. As already stated large excesses of alkali hydroxide and ammonia defer the end point of the titration on account of the slight solubility of silver cyanide in these alkaline hydroxides. When a few drops of potassium iodide are added to the solution this source of error may be wholly avoided. In the presence of this salt the end of the reaction is indicated by a turbidity due to silver iodide, a compound which is not soluble in dilute alkali hydroxide and ammonia solutions. The use of iodide allows ammonia to be used instead of fixed alkali for neutralising free hydrocyanic acid.

A simple method for the estimation of cyanogen when existing in the form of a cyanide of a light metal has been devised by Fordos and Gelis (*J. f. prakt. Chem.*, 1899, **59,** 255). The method is based on Serrulas and Woehler's reaction on the formation of cyanogen iodide:

$$KCN + I_2 = CNI + KI$$
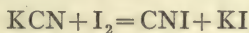
and gives under proper conditions fairly accurate results. Sulphides must be absent and any free alkalis or ammonia must be neutralised by addition of an excess of carbonated water.

2 grm. potassium cyanide are put in a 200 c.c. volumetric flask, which is filled to the mark and thoroughly shaken. 5 c.c. of this solution (0.05 grm. KCN) is taken and strongly diluted; now a $N/10$ solution of iodine is let in until a permanent yellow tint remains. A starch solution must not be used to determine the end reaction, as the results will then come too low. The number of c.c. $N/10$ iodine used multiplied by 26.04 gives % KCN.

In estimating solutions of hydrocyanic acid, the portion taken should not contain more than 0.05 grm. HCN, to which is added 4 c.c. normal sodium hydroxide solution, the excess of the sodium hydroxide being neutralised by the addition of about 0.5 grm. sodium hydrogen carbonate. The solution is now diluted to about 1,000 c.c. and titrated with $N/10$ iodine solution until a permanent yellow hue results. No starch must be used as indicator. 1 c.c. $N/10$ iodine solution = 0.005404 grm. HCN.

The gravimetric estimation of cyanogen in cyanides is carried out as follows: A weighed quantity of the cyanide is dissolved in water and is slightly acidified with nitric acid (any considerable excess of acid must be carefully avoided), and is then poured into a silver nitrate solution, when silver cyanide will be precipitated. The precipitate of

silver cyanide is allowed to settle without applying heat, when the liquid is filtered through a weighed filter, the precipitate washed, dried at 100° and weighed; the weight of the filter paper being deducted gives the weight of silver cyanide. If a grm. of product was originally weighed out and p grm. silver cyanide obtained then $\% \text{ KCN} = \dfrac{52.55p}{a}$.

The precipitate may be ignited in an open crucible for about 15 minutes and the residual metallic silver weighed, which should confirm the result first found. If a grm. substance is taken and p grm. silver found, then: $\% \text{ KCN} = \dfrac{60.35p}{a}$.

In the presence of chlorides, bromides and iodides the precipitated silver cyanide must be separated from the silver chloride, bromide and iodide. This can be conveniently accomplished by boiling the precipitate with a solution of mercuric acetate, in which the silver cyanide will dissolve, forming silver acetate and mecuric cyanide. The solution is then filtered, thus separating from the silver chloride, bromide and iodide. The silver in the filtrate is estimated by precipitating as silver chloride and then reducing the silver chloride in a current of hydrogen gas to silver. It is important to reduce the silver chloride to silver, instead of weighing the silver chloride direct, because when the silver is precipitated it is liable to bring down some mercury with it, which being enclosed in the precipitate does not volatilise by heating the silver chloride, which gives high results.

If sulphides are present in the cyanide then these must first be removed by agitating the liquid with freshly precipitated bismuth hydroxide, and then filtering off the same. Lead carbonate or white lead is generally used, but the author finds that the bismuth precipitate is easier and quicker to filter and wash out, thus making the treatment with freshly precipitated bismuth hydroxide more desirable.

Commercial Cyanide.

Commercial cyanide may be composed of potassium cyanide, sodium cyanide, chlorides, carbonates, hydroxides, cyanates, ferrocyanides, sulphides and cyanamides.

The cyanogen contents are estimated by Liebig's volumetric method. To estimate the potassium and sodium, about 0.5 grm. of the salt are dissolved in water, evaporated to dryness in a porcelain dish to which

5 c.c. dilute hydrochloric acid (20%) has been added; great precautions must be taken to evaporate under a hood which draws well, as hydrocyanic gas is evolved. The dry residue contains potassium and sodium chlorides, which are estimated in the usual way.

The chlorides in the cyanide can be estimated volumetrically with $N/10$ silver nitrate solution. Enough $N/10$ solution is added to produce permanent turbidity; then a further equal amount is added, which will precipitate all the cyanide present as silver cyanide. A few drops of potassium chromate are now added to the solution and the titration with $N/10$ silver nitrate is proceeded with until a pale red tint is observed which does not disappear on agitation. The last amount of $N/10$ silver nitrate used corresponds to the chlorides present.

The carbonates are estimated by adding an excess of barium nitrate to the solution of the cyanide, placing in a well closed flask and allowing the barium carbonate to settle. The barium carbonate is then filtered off and washed, precautions being taken to keep the air away as much as possible. From the amount of barium carbonate obtained, the CO_2 contents can be calculated.

Several methods have been suggested for the estimation of the hydroxides. It is very general to add magnesium nitrate to the filtrate of the barium carbonate precipitate, thus precipitating magnesium hydroxide which can be filtered off, washed, ignited and weighed as MgO, the amount found corresponding directly to the amount of hydroxides present.

Clennell (*The Cyanide Handbook*, 1910) suggests dissolving a weighed portion (about 1 grm.) in water, adding sufficient silver nitrate to give a permanent precipitate, then a few drops of an alcoholic solution of phenol-phthaleïn, and titrating with standard acid. The result obtained is equivalent to the hydroxide, plus that of half the carbonate, in terms of the standard acid employed. From this the amount of hydroxide may be calculated, the percentage of carbonate being already known. The Deutsche Gold- und Silberscheide Anstalt communicate that they use a neutral suspension of Prussian blue to estimate the hydroxides in cyanide. A 30% silver nitrate solution is added to the cyanide solution till a permanent turbidity is observed, then barium nitrate is added to precipitate the carbonates. Now, without filtering, the solution is heated to 30 to 40°, and while stirring titrated with a neutral suspension of Prussian blue, until the Prussian blue is not further converted into yellow prussiate and iron oxide; this

can be observed very distinctly as a blue hue is noticeable in the clear liquid after the precipitate has settled a little. If cyanates are present great care must be taken that the solution is not heated above 50°, as at higher temperatures alkali cyanates split up into ammonia and carbonates, which would react with the Prussian blue and give erroneous results.

The neutral suspension of Prussian blue is prepared as follows: Enough ferric chloride is added to an agitated solution of yellow prussiate of potash, leaving a slight excess of yellow prussiate. The precipitate is washed by decantation until it stops settling with ease. The neutral suspension of Prussian blue is shaken well and standardized against normal sodium hydroxide, and then diluted so that 10 c.c. decinormal sodium hydroxide is equivalent to about 10 c.c. of the suspension. The neutral suspension of Prussian blue keeps well and must be thoroughly shaken before filling the burette.

A method for the estimation of the hydroxides in cyanides used by the author consists in dissolving a weighed amount of the cyanide in water, adding silver nitrate until a permanent turbidity is obtained; then a little barium nitrate is added to precipitate the carbonates, without filtering; the solution is now titrated with a neutral solution of ferric chloride, using a few drops of potassium ferrocyanide solution as indicator. The dilute solution, which becomes light brown on the addition of neutral ferric chloride, takes a deeper brown colour as soon as an excess of a drop of ferric chloride is added. This method requires a little practice and gives good results. The neutral ferric chloride is standardised gravimetrically, and should be diluted so that 25 c.c. corresponds to about 0.2 grm. Fe_2O_3.

The most popular method in use for estimating the amount of alkali cyanate in cyanide is based on the solubility of silver cyanate in dilute nitric acid, while silver chloride and silver cyanide are insoluble. A known amount of alkali cyanide, about 0.5 grm., is dissolved in water, enough barium and magnesium nitrates are added to precipitate respectively the carbonates and hydroxides present. After the precipitates have settled they are filtered off and washed. A neutral solution of silver nitrate is added to the filtrate until no more precipitate is formed. The precipitate, which consists of silver cyanide, silver chloride and silver cyanate, is allowed to settle; it is then filtered off and washed until no more silver can be detected in the wash water. The precipitate is washed into a beaker and stirred up with about 200 c.c.

water to which 10 c.c. dilute nitric acid (sp. gr. 1.2) has been added. It is then digested on the water-bath for one hour. The beaker must be covered with a watch-glass and the contents frequently agitated. The silver cyanate will go into solution, while the other silver salts will remain insoluble. The solution is filtered off from the residue and in the filtrate the amount of silver is estimated gravimetrically by precipitating as silver chloride or more conveniently by Volhard's volumetric method. From the amount of silver gone into solution, the alkali cyanate present can be calculated. Unless the directions of this method are closely followed erroneous results will be obtained, because silver cyanide is appreciably soluble in dilute nitric acid, therefore, frequently unsatisfactory results are obtained.

A very desirable method for the estimation of alkali cyanates has been described by O. Herting (*Zeit. f. angew. Chem.*, 1901, **14**, 585). 0.2 to 0.5 grm. of the salt are placed in a porcelain dish and dissolved in a few c.c. of water. A little dilute hydrochloric or sulphuric acid is added and the dish is placed on a water-bath and evaporated to dryness. The residue is dissolved in water and the nitrogen content is estimated in this solution by distillation with sodium hydroxide, the distillate is received in $N/5$ sulphuric acid, the unneutralised portion being titrated with $N/5$ ammonia. The amount of nitrogen found is calculated to potassium cyanate.

To determine the amount of alkali ferrocyanide present, 0.5 grm. of the salt are dissolved in about 50 c.c. water and then 5 c.c. sulphuric acid added. The solution is evaporated in a platinum dish, proper precautions being taken to draw off the hydrocyanic gas evolved, the residue being gradually heated until it melts. As soon as the melt has cooled it is dissolved in water with a small amount of sulphuric acid; a few pieces of zinc are added to reduce any ferric sulphate present and the solution is then titrated with $N/10$ permanganate solution. 1 c.c. $N/10$ permanganate solution $=0.0369$ grm. potassium ferrocyanide. It is always advisable to run a parallel blank test with the same amount of sulphuric acid and zinc, and to deduct the amount of $N/10$ permanganate used.

Only a very small quantity of alkali sulphide is present in the commercial cyanide of to-day, and the same can be detected by adding a few drops of a lead salt to a solution of the cyanide; if sulphide is present a black precipitate is formed. It is best to use for this reaction a lead salt dissolved in an excess of alkali hydroxide.

The most convenient method for estimating the sulphides present (Ewan, *J. Soc. Chem. Ind.*, 1909, **28,** 10) consists in titrating a solution containing 10 grm. alkali cyanide with a standard lead nitrate solution, until a drop of the solution when brought in contact with a drop of lead nitrate solution, on heavy filter paper for drop reactions, no longer gives a brown stain.

The presence of cyanamide can be detected by adding an excess of strong ammonaical silver nitrate solution (10%) to a very dilute cyanide solution. If a trace of alkali cyanamide is present a yellow precipitate of silver cyanamide is formed. If a too concentrated solution of cyanide is used a beautiful white, crystalline scale-like precipitate of ammonium silver cyanide is formed. The same reaction is employed for the quantitative estimation of alkali cyanamide. The solution is made strongly ammoniacal, and then a slight excess of an ammoniacal solution of silver nitrate (10%) is added. The yellow precipitate of silver cyanamide (silver chloride and silver cyanide being soluble in ammonia) is filtered off, washed well and dissolved in dilute nitric acid. The silver in the solution is estimated by Volhard's volumetric method, *i.e.*, titrating the silver with $N/10$ potassium or sodium thiocyanate, using iron alum as indicator; a permanent red colouration shows the end point of the titration. 1 c.c. $N/10$ potassium thiocyanate used = 0.0059 grm. potassium cyanamide.

Analysis of Cyanide Solutions in Gold Works.

In the extraction of gold by the cyanide process, it is necessary for advantageous working to ascertain the strength of the dilute solutions of the potassium cyanide employed; however, for regulating the daily work of the plant only a few simple tests have to be made on the solutions. After repeated use the solutions are greatly reduced in strength, becoming contaminated with various impurities, some of which quite invalidate the ordinary methods of estimation. The presence of zinc is especially objectionable, since the potassium zinc cyanide cannot be readily determined and prevents a correct titration of the potassium cyanide either by the silver or iodine processes.

In the practical assay of such dilute cyanide liquors no method is admissible which does not give a perfectly definite end point and which is not easily and rapidly executed. The methods of analyses have recently been ably discussed in detail by J. E. Clennel ("The Cyanide Handbook," 1910, 438) of which the following is an epitome.

The daily routine tests consist of (1) estimation of free cyanide in the solutions entering and leaving the precipitation boxes, or in the sumps and storage tanks, before and after making up to the required strength, with occasional special tests in addition to these, (2) the estimation of the alkali hydroxides and (3) assays of gold and silver in the solutions entering and leaving the precipitation boxes.

In making the free cyanide test, solutions are frequently turbid from the presence of suspended matter. The solutions to be tested must be perfectly clear and if necessary they must be filtered. Only in a few exceptional cases can the clarifying be undertaken by agitation with lime. Such treatment with lime or any other substance is mostly inadmissible since it causes decomposition of the zinc cyanide, and thus raises the apparent strength of the solution. On the other hand, the decomposition it affects is always incomplete, so that it cannot be employed to eliminate the zinc. Dilution, addition of alkalis, variation of temperature and other conditions must also be avoided; all these affect the reading given by the silver nitrate, at least in the presence of zinc. The tests should be made in perfectly clean flasks and the reaction observed in a good light against a dark background, as the first turbidity, which marks the end point, is somewhat faint. From 10 to 50 c.c. of the solution, according to its strength in cyanide, are mixed with a few drops of strong solution of potassium iodide and titrated with a solution of silver nitrate, adjusted to give the result with little or no calculation. A strength commonly used is 13.04 grm. $AgNO_3$ per litre, in which case 1 c.c.=0.01 grm. KCN or 0.004 grm. CN. The end point is usually taken at the first appearance of a permanent yellowish turbidity, disregarding a slight white cloudiness which may appear earlier. This possibly gives slightly too high a result in presence of potassium zinc cyanide and its analogues, as some of the cyanogen of these compounds may be also indicated when free alkali hydroxide is present. This is not, however, of much consequence if the test be made in the same manner in all solutions. In absence of potassium iodide the end point (white turbidity) is reached sooner, but is generally uncertain and indefinite. An intermediate result, and one which, so far as tested, appears to correspond with the actual working strength of the solution, is obtained by neutralising the free alkali hydroxide and monocarbonates and then adding potassium iodide and titrating with silver nitrate in the usual way, taking as the end point the appearance of a distinct permanent white turbidity.

The result may be calculated in per cent. or in pounds or kilos per ton, as desired. If 10 c.c. of the solution to be examined has been taken, 1 c.c. of silver nitrate (13.04 grm. per litre) used = 0.1% KCN, 0.04% CN, 2 pounds KCN or 0.8 pounds CN per ton of 2,000 pounds, or 1 Kilogrm. KCN or 0.4 Kilogrm. CN per metric ton of 1,000 Kilogrm.

In the absence of zinc the alkali hydroxide is estimated as follows: Silver nitrate is first added to a measured volume of the cyanide solution to be tested until a permanent turbidity, or if the solution was originally turbid a distinct turbidity, is observed. Addition of a few drops in excess is immaterial. A drop of phenolphthaleïn solution is now added, and the liquid titrated with $N/10$ hydrochloric acid until the pink colour just disappears. The amount of $N/10$ acid used measures the alkali hydroxide.

In presence of zinc, before titrating with the acid, add a sufficient excess (about 10 c.c.) of a 5% solution of potassium ferrocyanide, and proceed exactly as above. The addition of ferrocyanide liberates the alkali, which would otherwise be precipitated as zinc hydroxide or carbonate on addition of silver nitrate, the reaction being probably somewhat as follows:

(1) $K_2Zn(CN)_4 + 2KOH + 2AgNO_3 = Zn(OH)_2 + 2KAg(CN)_2 + 2KNO_3$

(2) $2Zn(OH)_2 + K_4Fe(CN)_6 = Zn_2Fe(CN)_6 + 4KOH$
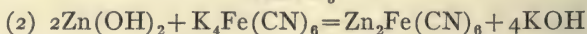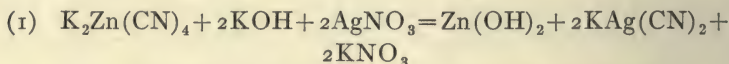
with analagous reactions in the case of carbonates. In some cases difficulties arise owing to the gradual return of the phenolphthaleïn colour on standing. This may be due to insufficient silver nitrate or ferrocyanide having been added, and may generally be avoided by adding the required amount of silver nitrate to precipitate all the cyanogen as silver cyanide, adding an excess of ferrocyanide and filtering before titrating the alkali. Any mineral acid or oxalic acid may be used for the standard solution. It is generally adjusted so as to give the alkalinity in terms of NaOH or CaO without further calculation. If a solution of oxalic acid ($C_2H_2O_4$, 2 aq.) containing 1.575 grm. per litre is used, then 1 c.c. acid = 0.001 grm. NaOH.

The total cyanide is defined by Clennell as "the equivalent in terms of potassium cyanide, of all the cyanogen existing as simple cyanides and easily decomposable double cyanides, such as $K_2Zn(CN)_4$" (excluding such substances as $KAu(CN)_2$, $Hg(CN)_2$, $K_4Fe(CN)_6$, KCNS, etc.). It is estimated by making the solution strongly alkaline

with sodium hydroxide, adding potassium iodide and titrating the cyanide with silver nitrate until a permanent yellowish turbidity is observed. For 50 c.c. of the solution to be tested it is usually sufficient to add 10 c.c. of an indicator containing 4% NaOH and 1% KI.

The best method of estimating the total cyanogen present in a solution appears to be to boil with oxide of mercury in excess, filter and remove mercury with an alkali sulphide. Any excess of sulphide is removed by agitating with lead carbonate, adding in small quantities at a time, and filtering. The clear liquid, after addition of potassium iodide, is titrated for cyanide in the ordinary way with silver nitrate. By this method practically all the cyanogen compounds are converted into mercuric cyanide, which is decomposed by the alkali sulphide into insoluble mercury sulphide and alkali cyanide.

To estimate the free hydrocyanic acid enough silver nitrate solution is added to form a permanent turbidity; should zinc be present then an excess of potassium ferrocyanide is added. The solution will now appear acid to phenolphthaleïn, and the amount of HCN present may be estimated by titrating this acidity with $N/10$ sodium hydroxide solution. 1 c.c. $N/10$ sodium hydroxide solution = 0.0027 grm. HCN.

Cyanogen bromide, as used in the Sulman, Teed and Diehl process, is estimated as follows: The solution is acidified with hydrochloric acid and potassium iodide added.

$$BrCN + HCl + 2KI = HCN + KCl + KBr + I_2.$$
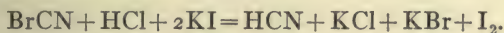
The liberated iodine is estimated in the usual way by titration with $N/10$ sodium thiosulphate. 1 c.c. $N/10$ thiosulphate = 0.0053 grm. BrCN. The presence of alkali cyanide does not interfere, as alkali chlorides are formed by the hydrochloric acid, hydrocyanic acid being evolved.

Ferrocyanides if present in large quantities are liable to give too high results by the silver nitrate titration of the cyanide; however, this is seldom the case and the error caused is unimportant.

In cases where all the iron in the solution exists in the form of ferrocyanide, the latter is best estimated by determining the total iron, after decomposition of the cyanogen compounds. This may be accomplished by using a powerful oxidizing agent, the iron then being precipitated by a slight excess of ammonia in the usual way.

Should much ferrocyanide be present then it is generally necessary to evaporate with nitric and sulphuric acids, sometimes more than

once, until the liquid, made alkaline and reacidified with hydrochloric acid, no longer shows a trace of blue colour. When this is the case the iron can be separated by ammonia and estimated in the usual way.

Should other soluble iron compounds be present, such as ferricyanides and nitroprusside, etc., it is best to estimate the ferrocyanogen anions together; then to estimate the ferricyanide, etc., in separate portions and estimate the ferrocyanide by difference.

If insoluble iron compounds exist in suspension and cannot be removed by filtration, the solution should be agitated with lime and filtered which will leave the soluble cyanogen compounds unaffected. If, however, the suspended matter itself contains insoluble ferrocyanogen compounds, they will be wholly or partially decomposed and a further quantity of ferrocyanogen added to the solution. In many cases Donath and Margosches' method (*J. f. prakt. Chem.*, 1899, **55**) may be used to great advantage. It is applicable for estimating ferrocyanide when iron is present in other forms and depends on the solubility of ferrocyanides in sodium hydroxide. The substance is first digested with 8% sodium hydroxide until as much as possible is dissolved by gently warming. It is then filtered and the filtrate treated with brominized sodium hydroxide, which is prepared by dissolving 20 c.c. of bromine in a little of the 8% sodium hydroxide. A precipitate of ferric hydroxide is thus obtained, representing only that part of the iron which was originally present as ferrocyanide. The ferric hydroxide is filtered off, preferably dissolved in hydrochloric acid and reprecipitated with ammonia.

Ferricyanides interfere very slightly with the correct titration of the cyanide by the silver nitrate method; however, instead of the white precipitate of silver cyanide ordinarily formed, a reddish brown precipitate of silver ferricyanide is produced, which becomes permanent on the completion of the reaction.

In the absence of other substances capable of liberating iodine from potassium iodide, ferricyanides may be estimated by the method of Lenssen and Mohr (Sutton, "Volumetric Analysis," 8th Edition, p. 227). After adding potassium iodide and acidifying with hydrochloric acid, add excess of zinc sulphate, allow to stand some time, and neutralise with sodium hydrogen carbonate. The liberated iodine is then titrated with $N/10$ thiosulphate in the ordinary way.

Ammonium thiocyanate may render the end reaction in the silver

titration somewhat obscure and is estimated by a colourimetric test. A known portion of the filtered solution is taken and acidified with hydrochloric acid, an excess of ferric chloride solution is then added and the mixture is shaken up with a small quantity of ether; the same amount of hydrochloric acid, ferric chloride solution and ether are brought to a like volume by the addition of distilled water and $N/10$ potassium thiosulphate solution is added till the red tint is the same as the tint formed by the solution.

It is occasionally necessary to estimate other organic compounds in the cyanide solutions, oxalates and urea forming the chief ones. C. J. Ellis (*J. Soc. Chem. Ind.*, 1897, **14,** 115) gives the following method for oxalates. The solution to be tested is precipitated with calcium chlorid in excess. The precipitate, after settling, is filtered off and washed, and dissolved in a small excess of hydrochloric acid. The oxalic acid thus formed is then titrated in warm solution by standard permanganate.

The urea may be determined by decomposition with sodium hypobromite, and warming slightly toward the finish. The nitrogen evolved is collected and measured in a suitable graduated tube, and the necessary correction for temperature and pressure applied, the carbon dioxide being absorbed in potassium hydroxide solution.

Determination of Metals in Cyanide Solutions.

All cyanogen compounds without exception (including ferrocyanides, ferricyanides and cobalticyanides) are completely decomposed, and the metals converted into sulphates or oxides, as the case may be, by treatment in platinum with a mixture of three parts concentrated sulphuric acid and one part water. In heating the mixture until nearly all the acid is expelled, the residual mass will be obtained free of cyanogen. It may be dissolved in water or acid, and the metals estimated by the usual methods. This method is not adopted for the use of mercuric cyanide, as some of the metal is volatilised.

The standard method, for the determination of gold, is that of evaporation with litharge. A measured quantity of the solution, usually not more than 300 c.c., is placed in a porcelain crucible. 20 to 50 grm. of litharge is then sprinkled over the surface of the liquid and the mixture allowed to evaporate at a gentle heat, without boiling. The evaporated residue is then fluxed as in an ordinary ore assay.

Several other methods for the estimation of gold in cyanide solutions

have been suggested, that of Christy (A. I. M. E., 1896, p. 1) is one of the best known. It consists in acidifying the solution and adding a copper salt together with a reducing agent, such as a soluble sulphite, whereby a precipitate of cuprous cyanide is formed which carries down the gold, leaving only a minute trace in solution. Whitby ("Proc. Chem. Met. and Min. Soc. of S. A.," **3,** 15) has slightly modified this method; he adds first a sufficient volume of 10% copper sulphate, then a few c.c. concentrated hydrochloric acid and finally 10 to 20 c.c. of a 10% sodium sulphite.

A satisfactory method, for the determination of gold in electro-gilding solution, consists in taking a measured quantity of the gilding solution, placing it in a porcelain dish and carefully concentrating same. When a syrupy consistency is obtained a few grm. pure red lead or litharge are added, the evaporation being then continued to complete dryness. The crucible is covered and brought for a short time to a moderate red heat. The lead oxide is reduced by the cyanide present, forming metallic lead and alkali cyanate, the reduced metal uniting with the gold and forming a button. The button is separated from the slag and the gold determined by cupellation. The precious metals found in an electrodepositing liquid are generally reported in Troy ounces, pennyweights and grains per pint of solution.

Silver is determined by the same methods as described under gold. In dilute solution, the silver may be separated, free from gold, by precipitating with sodium sulphide after adding a few drops of a solution of some lead compound. The precipitate should be washed by a dilute solution of sodium sulphide, dried, scorified and cupelled, or it may be converted into bromide by the addition of bromine, washed, dried, fused and weighed as silver bromide.

A method, for the estimation of metallic silver in electroplating solutions, consists in taking a measured quantity of the liquid, heating same to boiling and then passing hydrogen sulphide through, or else to gradually add ammonium sulphide. The silver falls as silver sulphide and is liable to be contaminated with zinc and copper. The precipitate is filtered, washed and then washed into a flask or beaker, and treated with an excess of bromine-water. It is thus rapidly and completely converted into silver bromide. If any sulphur appears to have separated a few drops of bromine should be added to ensure complete oxidation. The contents of the flask are diluted with water and boiled. The precipitate is now filtered, washed, dried and weighed.

For the estimation of zinc, the solution is made strongly alkaline with sodium hydroxide, heated to boiling and sodium sulphide added as long as a precipitate forms. The precipitate is filtered and washed, and may contain besides zinc silver, mercury or lead. All these metals may be determined by any suitable method. If the zinc is alone required then take the well washed precipitates of sulphides and transfer to a flask together with the filter paper. Now add $N/10$ iodine in excess and some very dilute hydrochloric acid.

$$ZnS + 2HCl + I_2 = ZnCl_2 + 2HI + S.$$
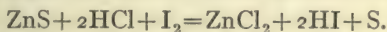
Air should be excluded as much as possible. The contents of the flask are well shaken, allowed to stand for a few minutes and the unused iodine titrated with $N/10$ potassium thiosulphate.

For the estimation of copper it is best to decompose the cyanogen compounds by boiling the solution with hydrochloric acid and potassium chlorate and then precipitating the copper by any of the known methods.

The bad extraction of gold is sometimes due to the presence of reducing agents in the cyanide solutions. The test for the reducing power is generally estimated by acidifying the solution and titrating with $N/100$ potassium permanganate, until a permanent pink colour is obtained.

A better method is to add to the acidified solution an excess of permanganate, and after standing for some time till the reaction is complete, to estimate the excess by adding potassium iodide and titrate the liberated iodine with sodium thiosulphate. Fixed quantities of acid, solution and permanganate should be used in all tests. If this estimation be made at regular intervals, any variations from normal conditions will be at once detected.

It is frequently necessary to ascertain the "solvent activity" of a cyanide solution, *i.e.*, the efficiency of a cyanide solution for dissolving gold (J. E. Clennell, *Eng. and Min. J.*, 1904, **77**, 513). The most satisfactory method is to prepare equal quantities of precipitated gold in a number of separate and similar vessels by means of gold chloride and sulphurous acid, making faintly alkaline with sodium hydroxide and adding to each a fixed volume of the various solutions to be compared. After agitating a definite length of time, the residual gold is filtered off, dried, cupelled and weighed. The difference between this weight and that of the gold taken is the measure of the solvent activity of the solution.

Double Cyanides.

As already stated, the cyanides of the heavy metals exhibit a remarkable tendency to form double salts which in many instances are of an exceedingly stable nature, so that in some cases neither the cyanogen nor the heavy metal is recognisable by any reaction which does not involve actual destruction of the compound cyanide.

Such stable double cyanides are not precipitated by alkalis or decomposed by carbonic acid, and their aqueous solutions are usually quite neutral in reaction. On ignition without access of air they are decomposed, and the cyanide of the alkali metal may then be dissolved out from the residual heavy metal or metallic carbide by means of water.

Many of the double cyanides are decomposed on addition of a dilute mineral acid, as in the following instance:

$$KAg(CN)_2 + HNO_3 = AgCN + HCN + KNO_3.$$
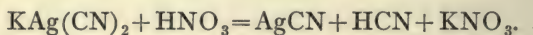
The liberated hydrocyanic acid has, in such cases, no tendency to combine with the cyanide of the heavy metal.

Double cyanides which suffer decomposition in the above manner respond to the tests for simple cyanides (page 477), except that the precipitate produced by silver nitrate in a neutral solution, is not pure silver cyanide, but a mixture or compound of the two cyanides of the heavy metals, as in the following instance:

$$2KCN,Zn(CN)_2 + 2AgNO_3 = 2AgCN,Zn(CN)_2 + 2KNO_3.$$
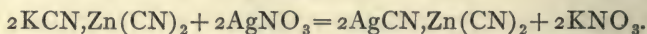
On treating the precipitate with dilute nitric acid, the zinc cyanide dissolves and the silver cyanide remains.

Of the really decomposable double cyanides, those of mercury, silver, zinc and cadmium are decomposed by hydrogen sulphide readily and completely, with precipitation of the corresponding metallic sulphides. Most of the other double cyanides of this class (e.g., copper and nickel) are decomposed very imperfectly or not at all.

Other of the double cyanides are of a still more stable character, and on treatment with a dilute mineral acid, the liberated hydrocyanic acid remains in close combination with the cyanide of the heavy metal forming a new compound acid giving rise to a complete and characteristic series of salts. The following equation represents the action of hydrochloric acid on potassium ferrocyanide:

$$K_4Fe(CN)_6 + 4HCl = 4KCl + H_4Fe(CN)_6 \text{ (Hydroferrocyanic Acid).}$$
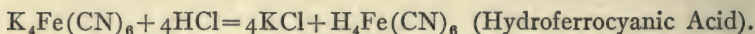
From the foregoing description it is evident that the double cyanides may be conveniently arranged in two classes, namely: (1) those which are readily decomposed by dilute mineral acids, and (2) those which are not materially affected by such treatment. The more important salts of Class 1 are described below, while the principal stable double compounds are considered in separate sections.

Readily Decomposable Double Cyanides.

These compounds have a great practical interest from their application in the treatment of gold ores and in electro-metallurgy. The estimation of the metals contained in them is described on page 493, and of the cyanide on page 490.

Potassium Zinc Cyanide, $2KCN, Zn(CN)_2$, is the type of the readily decomposable double cyanides. It has a practical interest as a convenient source of free hydrocyanic acid, as a suitable compound for the electro-decomposition of zinc, and as a constituent of the cyanide liquors of gold-extraction works.

Potassium zinc cyanide is readily prepared by precipitating a solution of zinc sulphate or chloride with an equivalent amount of potassium cyanide, and dissolving the washed precipitate of zinc cyanide in a solution of a second equivalent of potassium cyanide. On concentration, the solution deposits large, colourless, regular octahedra of the double cyanide. The salt is fusible, permanent in the air, and very soluble in water to form a solution of a sweet taste. Addition of a moderate quantity of acetic, hydrochloric or sulphuric acid to the solution precipitates zinc cyanide, which redissolves in excess of the precipitant.

The solution of potassium zinc cyanide has an alkaline reaction to litmus. With methyl orange the neutral point is stated to correspond with the complete conversion of the potassium and zinc into chlorides:

$$K_2Zn(CN)_4 + 4HCl = 2KCl + ZnCl_2 + 4HCN.$$
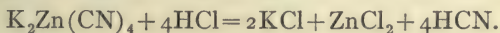
With phenolphthalein the neutral point is reached, when sufficient has been added for the reaction:

$$10K_2Zn(CN)_4 + 3HCl = 3HCN + 3KCl + (Zn(CN)_2)_{10}(KCN)_{17}.$$
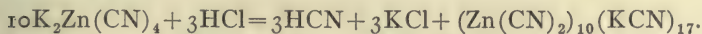
On addition of excess of potassium hydroxide to the solution of potassium zinc cyanide, potassium cyanide and zincate are formed according to the equation:

$$K_2Zn(CN)_4 + 4KOH = 4KCN + K_2ZnO_2 + 2H_2O.$$
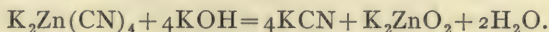
Sodium hydroxide acts similarily, as also do potassium and sodium carbonates, but acid carbonates have no action.

Sodium sulphide or hydrogen sulphide throws down the whole of the zinc from a solution of potassium zinc cyanide. This reaction affords a means of separating zinc from nickel and copper, the sulphides of which are not similarly precipitated. The zinc contained in the cyanide liquors of gold works may also be estimated in the manner described on page 495.

Potassium Copper Cyanide exists in several different molecular proportions, of which potassium cuprous cyanide, $K_2Cu(CN)_4$, is the best known. This potassium cuprous cyanide is formed when a solution of cupric sulphate is added in limited quantity to potassium cyanide; thus a yellowish-brown precipitate of cupric cyanide, $Cu(CN)_2$, is obtained. On boiling the liquid this suffers more or less complete reduction to white cuprous cyanide, $Cu_2(CN)_2$, cyanogen being evolved as gas. Cuprous cyanide dissolves in potassium cyanide solution to form a colourless liquid containing potassium cuprous cyanide, $2KCN$, $Cu_2(CN)_2$, which can be obtained in colourless crystals on evaporation. The solution is not precipitated by alkalies nor by hydrogen sulphide, a fact utilised in analysis to separate copper from cadmium.

A solution of ammonium cupric sulphate becomes colourless or faintly yellow on addition of potassium cyanide, a fact on which is based Parke's process for the volumetric estimation of copper.

The copper is not precipitated from a solution of potassium cuprous cyanide by contact with iron, a fact which is utilised in the electro-deposition of copper on iron surfaces.

If potassium cyanide be added to Fehling's boiling copper solution, the blue colour is destroyed as in the last case, and the liquid gives no precipitate of cuprous oxide when boiled with glucose; but if Fehling's solution be present in quantity more than sufficient to react with the potassium cyanide used (*i.e.*, if the mixed solutions retain a blue colour), this extra portion will suffer a reduction by glucose; but instead of the cuprous oxide being precipitated it will remain in solution, and the progress and the end of the reduction will be indicated by the gradual lessening and ultimate entire disappearance of the blue colour.

Potassium Silver Cyanide, KCN, AgCN, is obtained by precipitating silver nitrate by an equivalent amount of potassium cyanide, and dissolving the white curdy silver cyanide thus obtained in a second

equivalent of potassium cyanide solution. On evaporation, the solution deposits hexagonal tables of potassium silver cyanide. The compound may also be obtained by the action of potassium cyanide solution on metallic silver in the presence of air, by dissolving silver chloride in potassium cyanide solution, or by adding potassium cyanide to silver nitrate solution until the precipitate at first formed is redissolved.

A solution of potassium silver cyanide is decomposed on addition of a dilute mineral acid with evolution of hydrocyanic acid and precipitation of silver cyanide. From a dilute solution the silver is completely thrown down by hydrogen sulphide or ammonium sulphide. Any zinc present will accompany the silver, but nickel and copper will remain in solution.

Potassium silver cyanide is extensively used for electroplating. The contained silver may be estimated as described on page 490, and the cyanogen as described on page 494.

Potassium Gold Cyanides.—On adding potassium cyanide to a solution of auric chloride, a precipitate of auric cyanide, $Au(CN)_3$, is first obtained, but this dissolves with great facility in excess of the reagent with formation of potassium auric cyanide, $KCN, Au(CN)_3$, which on evaporation can be obtained in colourless crystals containing $1H_2O$. The solution is used for electrogilding.

A solution of potassium cyanide readily dissolves gold if access of air be permitted, the reaction apparently being:

$$2Au_2 + 8KCN + 2H_2O + O_2 = 4(KCN, AuCN) + 4KOH.$$
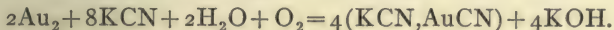
On evaporation the solution yields colourless octahedral crystals of potassium aurous cyanide.

The foregoing reaction is the foundation of the cyanide process of gold extraction now so extensively used for the treatment of gold ores. The quantity of cyanide theoretically necessary to dissolve a given weight of the gold is very small in comparison with the weight required in practice, which is at least 40 parts of cyanide for 1 of gold. This is due in the first place to instability of potassium cyanide, which is decomposed by atmospheric carbon dioxide with formation of hydrocyanic acid and potassium carbonate. Further loss takes place by oxidation of the cyanide to cyanate and carbonate, and in the presence of the excess of sodium or potassium hydroxides much loss

results from hydrolysis with formation of potassium formate and ammonia. More or less decomposition of the cyanide also probably occurs in dilute solutions, with formation of hydrocyanic acid and potassium hydroxide:

$$KCN + H_2O = HCN + KOH.$$
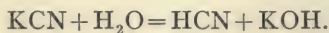
Evidence of this reaction is afforded by the fact that on passing nitrogen or other inert gas through a cold dilute solution of potassium cyanide, hydrocyanic acid is volatilised, and the fact accounts for the odour of hydrocyanic acid always perceptible in the vicinity of cyanide tanks freely exposed to the air. A further loss of cyanide occurs from the formation of ferrocyanides and analogous compounds. The appearance of a blue colouration on the surface of the tailings or in the cyanide solution is a certain indication that acid salts of iron are present, and that a large loss of cyanide has occurred.

Traces of free sulphuric acid exist in pyritous tailings, and cause evolution of hydrocyanic acid.

Ferrous sulphate acts on potassium cyanide with formation of a yellowish-red precipitate of ferrous cyanide, which combines with more potassium cyanide to form ferrocyanide, $4KCN, Fe(CN)_2$, and in presence of free acid Prussian blue is formed. Ferric salts, in the absence of ferrous salts, decompose potassium cyanide with evolution of hydrocyanic acid and precipitation of ferric hydroxide in a finely divided colloidal condition, with difficulty removed by filtration. Mixed ferrous and ferric sulphates, which are probably always present in partially oxidised pyritic tailings, cause a blue colour on addition of the cyanide, after the free alkali of the commercial product has been neutralised.

Before treating such pyritic ores or products with cyanide, it is necessary to subject them to a treatment with water to remove free acid and soluble salts of iron, followed by washing with a solution of sodium hydroxide or lime to decompose the basic sulphates. Slaked lime is sometimes mixed with the tailings before commencing the cyanide treatment.

Lime is preferable to soda, as though slower in its action it decomposes the iron salts equally well, is less active in effecting hydrolysis of the cyanide, and is less energetic in attacking the zinc in the precipitating tanks.

Ferric hydroxide does not appear to be acted on by potassium cyanide, but ferrous hydroxide reacts with excess of potassium cyanide to form ferrocyanide:

$$Fe(OH)_2 + 6KCN = 4KCN, Fe(CN)_2 + 2KOH.$$

The gold contained in the cyanide liquor is precipitated by metallic zinc, sodium amalgam or by electrolysis.

Zinc is used in the form of clean turnings, and appears to act on the aurous potassium cyanide according to the following equation:

$$2KAu(CN)_2 + Zn = K_2Zn \ (CN)_4 + Au_2.$$
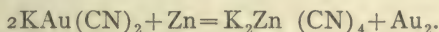
Hence theoretically 1 pound of zinc should precipitate about 6 pounds of gold, but in practice the actual consumption of zinc is about 1 pound for every Troy ounce of gold precipitated. Other cyanides of the light metals can be substituted for potassium cyanide.

Reduction of the dissolved gold by sodium amalgam instead of zinc presents many advantages, since the solutions do not become saturated with zinc compounds, and the whole of the cyanogen is restored to a condition in which it is available for dissolving gold. The amalgam may be made by direct union of sodium and mercury, but in the Molloy process it is produced electrolytically.

In the Molloy process the cyanide solution passes through a shallow trough containing mercury, in which trough is an inner cylindrical vessel filled with a solution of sodium carbonate. The edges of the cylinder just dip beneath the mercury, so that the contents are entirely cut off from the outer portion of the vessel. A rod of lead dips into the sodium carbonate solution and forms the anode, the lead and mercury being connected with opposite poles of a battery, so that the sodium carbonate is electrolysed by the current. The nascent sodium combines with the mercury to form an amalgam, which at once reduces the gold in the cyanide solution to form ordinary gold amalgam and sodium cyanide, which salt is equally efficacious with potassium cyanide as a solvent for gold.

H. S. Sulman (*J. Soc. Chem. Ind.*, 1895, **14,** 753) has proposed to use of cyanogen bromide for renovating the cyanide solutions. (See page 461.)

In the process of Siemens and Halske, the gold is deposited electrolytically, very thin lead plates being used as the cathode and iron plates as the anodes. Prussian blue is formed by the action of the dissolved

iron on the ferrocyanides produced in the leaching, and is decomposed by alkali, the solution is evaporated, and the cyanide recovered by fusion with sodium carbonate. Siemens and Halske's process works well with cyanide solutions of any strength, even in the presence of sodium hydroxide, and hence renders possible the employment of very weak cyanide liquor for the extraction of the gold, thus taking advantage of the selective action and avoiding the simultaneous solution of copper.

From the waste solutions which have passed the zinc boxes, or been treated by Siemens and Halske's process, a further quantity of gold can be recovered by addition of zinc dust, preferably freed from oxide by means of ammonia.

Compounds of Cyanogen and Iron.

The affinity of cyanogen, hydrocyanic acid and other volatile or gaseous compounds of cyanogen to certain ferrous compounds, especially ferrous hydroxide, forming simple and complex iron and cyanogen compounds, renders some of these compounds of great commercial and industrial value.

Iron forms two sets of compounds with cyanogen, ferrous cyanogen compounds, and ferric cyanogen compounds according to whether the cyanogen compound reacts with a ferrous oxide (FeO) or a ferric oxide product (Fe_2O_3).

Of the simple iron cyanides only ferrous cyanide, $Fe(CN)_2$, is known, ferric cyanide $Fe_2(CN)_6$, not having yet been isolated in a free state.

Ferrous cyanide is formed by precipitation from a ferrous sulphate solution by potassium cyanide solution. It is a white amorphous product, which easily oxidises in the air forming a blue colour. It is easily soluble in alkali hydroxides and carbonates, forming characteristic double cyanides, which differ from the previously described double cyanides by their stable properties. Neither the iron nor the cyanogen can be detected as such in these double cyanides, as they form together the anion $Fe^{II}\!\!\begin{array}{c}(CN)_3 = \\ (CN)_3 = \end{array}$. The bond of iron is so strong in this anion that concentrated hydrochloric acid will not destroy it. Ferrocyanide salts, when boiled with dilute hydrochloric and sulphuric acids, liberate only a part of the hydrocyanic acid, the iron always remaining strongly bonded to the cyanogen.

By oxidising the ferrocyanide salts with chlorine, bromine, mag-

nesium peroxide, etc., ferricyanide compounds are formed in which the iron is tervalent $Fe^{III} \begin{subarray}{l} \diagup (CN)_3 \\ \diagdown (CN)_3 \end{subarray} =$, here also the iron and cyanogen are strongly bonded together forming an anion. The ferricyanide salts can be easily converted again to ferrocyanide salts by reducing agents. The alkali, ferro- and ferricyanides are easily soluble in water; earth alkali and magnesium compounds of ferro- and ferricyanide are somewhat soluble in water; other metallic ferro- and ferricyanides are insoluble in water, many of the latter showing characteristic colours.

Ferrocyanides.

Ferrocyanides are present in considerable proportion in the spent oxide and ammoniacal liquor of the gas works. Great attention has been given of recent years to the economical production of ferrocyanides, as a link in the manufacture of cyanides for gold extraction, etc., though their direct employment for the preparation of Prussian blue is a decaying industry. Hydroferrocyanic Acid, $H_4Fe(CN)_6$, can be precipitated from a cold saturated solution of potassium ferrocyanide by slowly adding hydrochloric acid. The acid is soluble in alcohol, from which it can be precipitated by the addition of ether. If dried in a current of hydrogen at 90°, it forms a snow-white crystalline mass, which is stable in the air.

Hydroferrocyanic acid has strong acid properties, combining with all bases to the corresponding salt; 100 parts of water dissolve 15 parts of the acid at 14°. By gently heating the acid, hydrocyanic acid is evolved and a blue residue remains. The acid heated in water splits up into ferrous cyanide and hydrocyanic acid. Ferrous cyanide is stable up to about 450°, when it gradually breaks up forming iron carbide and nitrogen.

Potassium Ferrocyanide, $K_4Fe(CN)_6 + 3H_2O$ (also known as "yellow prussiate of potash" (German, gelbes Blutlaugensalz; French, Prussiate jaune de potasse), on account of its colour, is possibly the best known and one of the most used of the iron and cyanogen compounds. It is formed by passing hydrocyanic acid through a suspension of ferrous hydroxide in a solution of potassium hydroxide or carbonate, or else by digesting ferrocyanide compounds with potassium hydroxide.

Potassium ferrocyanide is still sometimes manufactured by heating

carbonate of potassium with horns, hoofs, dried blood, wool and hair clippings, feathers, leather-parings or other animal refuse. Scrap iron is sometimes added, but in other cases the manufacturer relies on the iron of the vessel employed, which is made of great thickness on purpose. This wear of the iron pots, aggravated by the presence of sulphur in the horns, etc., used, and by the high temperatures necessary for the reaction, renders the life of the prussiate pots a very short one. The fused mass obtained, called "metal," is lixiviated with water and a clear liquid is obtained, which contains cyanogen equivalent to about 16 to 20% of potassium ferrocyanide, which, before lixiviation, may exist as cyanide, but on treatment with water, a double decomposition occurs, thus:

$$13KCN + Fe_2S_3 = 2K_4Fe(CN)_6 + 2K_2S + KCNS.$$
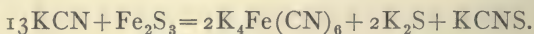
The insoluble residue from lixiviation consists largely of carbon derived from the animal matters, and used to be a waste-product. It was recently of value as a substitute for animal charcoal for the decolourisation of paraffin-wax and oils. The clear liquid from the lixiviation is boiled down, when a first crop of crystals separates. These crystals are then purified by recrystallisation. If the potassium carbonate contains more than about 3% sodium carbonate, the crystallisation is interfered with, the first crop of crystals (or "prussiate scale") contains about 97% hydrated ferrocyanide, and on recrystallisation it is obtained with 99.8%. The usual impurities are sulphate, sulphite, sulphide, thiosulphate (hyposulphite), chloride and carbonate of potassium. Very considerable quantities of sulphate are sometimes present, which may be detected and estimated by barium chloride. In addition to these impurities, the mother liquors contain sodium salts, thiocyanate (sulphocyanide), silicate, etc.

In the laboratory potassium ferrocyanide can be prepared by dissolving ferrous hydrate or freshly precipitated ferrous sulphide in potassium cyanide solution.

Potassium ferrocyanide forms amber-yellow monoclinic crystals, containing three molecules of constitutional water, the whole of which is expelled at 100°. It is not poisonous, but reacts on the human body as a purgative. It is claimed to have a poisonous action on plant life. The physiological action of potassium ferrocyanide on animals shows that this salt is not poisonous, even when given to animals in doses of 2 grm. per kilogrm. of body-weight. In those animals which do not

vomit, a diuretic action is observed three hours after the administration even in small doses. Repeated doses of the salt cause internal troubles in the dog, and vomiting ensues if the dose given exceeds 0.08 grm. per kilogrm. of body-weight. It is pointed out by the observers that potassium ferrocyanide, in its passage through the body, is transferred into ferricyanide, and as such is eliminated in the urine. The investigators suggest that its diuretic action may be due to this transformation.

Potassium ferrocyanide is tolerably stable at ordinary temperatures, both in the solid state and in solution. The salt is soluble in water, but is insoluble in alcohol. It has a perfectly neutral reaction to litmus, methyl-orange, and phenolphthaleïn. Dilute acids liberated hydroferrocyanic acid from potassium ferrocyanide. By moderately dilute hot sulphuric acid the salt is decomposed with evolution of hydrocyanic acid. When heated with excess of strong sulphuric acid, carbon monoxide is evolved as gas, and potassium, ammonium and iron sulphates are formed:

$$K_4Fe(CN)_6 + 6H_2SO_4 + 6H_2O = 6CO + 2K_2SO_4 + 3(NH_4)_2SO_4 + FeSO_4.$$
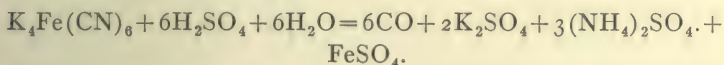
100 parts water dissolve 35 parts of potassium ferrocyanide ($K_4Fe(CN)_6 + 3H_2O$) at 15° and 100 parts at 100°. Schiff (*Ann. Chem. Pharm.*, 1877, **113,** 199) has determined the sp. gr. of different strength solutions at 15°, as is given in the following table:

In 100 Parts of Solution.		
$K_4Fe(CN)_6 + 3H_2O$	$K_4Fe(CN)_6$	Sp. Gr. 15°
1	0.872	1.0058
2	1.744	1.0116
3	2.618	1.0175
4	3.488	1.0234
5	4.360	1.0295
6	5.232	1.0356
7	6.104	1.0417
8	6.976	1.0479
9	7.848	1.0542
10	8.720	1.0605
11	9.592	1.0669
12	10.464	1.0734
13	11.336	1.0800
14	12.208	1.0866
15	13.080	1.0932
16	13.952	1.0999
17	14.824	1.1067
18	15.696	1.1136
19	16.568	1.1205
20	17.440	1.1275

Potassium ferrocyanide can be dehydrated by gentle heating, when a white triturable mass is obtained. Heated in the air potassium cyanate and iron oxide are formed, while potassium cyanide, iron carbide and nitrogen are formed when ignited in a closed vessel. Mixed with chlorates or nitrates and then heated it explodes violently, hence its use in the explosive industry.

Sodium ferrocyanide, $Na_4Fe(CN)_6 + 10H_2O$, is prepared similarly to potassium ferrocyanide and has the same properties. Its solubility according to Conroy (*J. Soc. Chem. Ind.*, 1898, **17**, 103) is:

Temperature	$Na_4Fe(CN)_6 + 10H_2O$ in 100 c.c.
18°	2.945
20°	3.185
42°	5.85
53°	7.59
58°	8.84
60°	9.02
77°	12.95
80°	14.60
96°	15.70
98°	15.75
98.5°	16.10

Ammonium ferrocyanide, $(NH_4)_4Fe(CN)_6 + 3H_2O$, is prepared by neutralising hydroferrocyanic acid with ammonia. This solution gives off ammonium cyanide when boiled. Its properties are similar to potassium ferrocyanide.

There are numerous complex compounds of ferrocyanide salts which may contain the alkali metals, and moreover alkali ferrocyanides crystallise easily together with other salts, especially with alkali nitrates.

Earth alkali ferrocyanides are prepared by treating hydroferrocyanic acid with an equivalent of the alkali earth hydroxide. If the alkali ferrocyanide solutions are treated with alkali earth salts, insoluble double compounds of alkali earth alkali ferrocyanides are formed. Alkali earth ferrocyanides are soluble in water.

Prussian Blue is ferric ferrocyanide, $Fe_4(Fe(CN)_6)_3$. The commercial product, however, is, or may be, a mixture of true Prussian blue with Turnbull's blue, Williamson's blue, and possibly other cyanides of iron. The following table shows the relationship of these and allied compounds:

$Fe_3(CN)_6$	Ferrous ferrocyanide	$Fe_2^{II}(Fe(CN)_6)^{IV}$
$K_2Fe_2(CN)_6$	Potassium ferrous ferrocyanide, Everitt's salt	$K_2Fe^{II}(Fe(CN)_6)^{IV}$
$KFe_2(CN)_6$	Potassium ferric ferrocyanide,[1] Williamson's blue	$KFe^{III}(Fe(CN)_6)^{IV}$

[1] Which of the formulæ given for Williamson's blue is correct is uncertain and the problem is apparently beyond solution.

$K_2Fe_4(CN)_{12}$	Dipotassium diferric diferrocyanide, soluble Prussian blue	$K_2Fe_2^{III}(Fe(CN)_6)_2^{IV}$
$Fe_7(CN)_{18}$	Ferric ferrocyanide, Prussian blue	$Fe_4^{III}(Fe(CN)_6)_3^{IV}$
$KFe_2(CN)_6$	Potassium ferrous ferricyanide,[1] Williamson's blue	$KFe^{II}(Fe(CN)_6)^{IV}$
$Fe_5(CN)_{12}$	Ferrous ferricyanide, Turnbull's or Gmelin's blue	$Fe_3^{II}(Fe(CN)_6)_2^{III}$

$KFe^{III}(Fe(CN)_6)^{IV}$ and $KFe^{II}(Fe(CN)_6)^{III}$ have the same ultimate composition, and yield the same products on treatment with alkalies. Soluble Prussian blue seems to be isomeric with Williamson's blue.

Prussian blue is the most important of these compounds and was discovered by Diesbach in 1704, and was the first known cyanogen compound.

Prussian blue is prepared by adding soluble ferric salts to solutions of ferrocyanide salts, forming ferric ferrocyanide $Fe_4(Fe(CN)_6)_3$. In the laboratory it is prepared by adding a solution of potassium ferrocyanide to a solution containing an excess of ferric chloride. The mixture is boiled to gather the precipitate and make it flocculent, when it is filtered, washed with hot water and dried at 100°. When dry, it forms a dark blue amorphous substance, having a strong coppery lustre and conchoidal fracture. The characteristics of good Prussian blue are lightness; and a deep, fine, blue colour. A coppery lustre is usual, but not an essential character. Prussian blue should adhere strongly to the tongue. It should not effervesce with acids, or thicken when boiled with water.

It is insoluble in water, alcohol and dilute mineral acids, but is easily soluble in oxalic acid solutions. According to Schoras (*Ber.* 1870, **3,** 13) the Prussian blue is again suddenly precipitated from the oxalic acid solution when placed in the sunlight. Coffignier (*Bull. Soc. Chim.,* 1902, **27,** 696) found that a mixture of equal parts concentrated hydrochloric acid and alcohol dissolves Prussian blue, forming a colourless liquid. Watson Smith (*J. Soc. Chem. Ind.,* 1903, **22,** 472) found that the solubility increases with the higher molecular weight of the alcohol used. According to Fresenius and Gruenhut (*Neuste Erfahrungen und Erfindungen,* **27,** 179) solutions of Prussian blue can be obtained in ether and chloroform, if it is first ground in a dry condition with oleic acid or fatty oils.

Prussian blue always contains water and is very hygroscopic. At 40° it contains about 28% water, and at 100° it loses part of this water,

but only loses all its water at 240°, at which temperature the dry product starts to decompose.

Prussian blue heated with alkali hydroxides forms quantitatively alkali ferrocyanide and ferric hydroxides. If boiled with mercuric oxide, mercuric cyanide and ferric hydroxide are formed.

Prussian blue is often adulterated with alumina, starch, barium sulphate, calcium carbonate, etc. The last may be detected by the effervesence on addition of dilute hydrochloric acid. In the solution obtained by digestion with the acid for some time, alumina may be detected by the addition of ammonia. Starch may be detected by boiling the sample with water, which will produce paste in the presence of a large proportion. Smaller quantities may be detected by digesting the finely powdered sample with magnesia and water in the cold; the residue is filtered and washed, and treated with cold dilute hydrochloric acid. The oxide of iron and excess of magnesia are dissolved, and the residual starch can be weighed, examined under the microscope, and tested with iodine. Any china clay or barium sulphate will remain with the starch, and, after removing the latter by ignition or boiling with water, the residue may be examined with a view to their recognition.

Prussian blue is manufactured in a great variety of shades. The lighter are useful for the manufacture of zinc greens, while the darker are employed for the manufacture of chrome greens. According to J. C. Gentele, the best blues are obtained by treating, in the first instance, a ferrous salt with yellow prussiate, and then oxidizing the bluish-white precipitate of ferrous ferrocyanide. This may be affected by nitric acid, bleaching powder and hydrochloric acid, etc.

The pigment which in German commerce goes by the name of "Prussian blue" is Paris blue (i.e., the best quality of ferrocyanogen blue) mixed with starch, barium sulphate, gypsum, burned and finely ground kaolin, or other dilutents. Very low varieties of blue are often "faced" by making the dried blue rotate in a cask charged with fine dust of pure Paris blue.

The proporion of real ferrocyanide contained in Prussian blue may be determined by treating the sample with sodium hydroxide, filtering from the iron oxide, and determining the ferrocyanide in the filtrate as described on page 510.

The colouring power of Prussian blue may be tested by grinding the sample with a large proportion of white-lead and oil, and comparing the colour with that given by a standard sample of known purity.

Detection of Ferrocyanides.

Insoluble salts of hydroferrocyanic acid, when treated with alkali or alkali earth hydroxides, are converted into the corresponding alkali or alkali earth ferrocyanide, a soluble salt, when they will respond to the following tests:

Dilute sulphuric acid does not react on ferrocyanide salt solutions, except at boiling temperature when hydrocyanic acid is evolved.

Concentrated sulphuric acid reacts with ferrocyanides evolving carbon monoxide gas.

$$K_4Fe(CN)_6 + 6H_2SO_4 + 6H_2O = FeSO_4 + 2K_2SO_4 + 3(NH_4)_2SO_4.$$
$$+ 6CO.$$
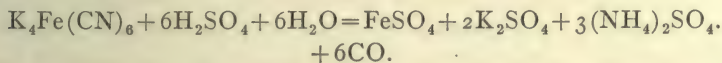
Carbon monoxide is detected by the characteristic blue flame by which it burns, and this reaction can be used for making a pure carbon monoxide in the laboratory. It is true that sulphur dioxide is liberated on account of part of the ferrous sulphate being oxidised to ferric sulphate, but this impurity can be easily removed by absorption in alkali hydroxide.

Almost any oxidising agent, chlorine, bromine, hydrogen peroxide, permanganate, and other per-salts, etc., will convert ferrocyanides easily into ferricyanides.

Silver nitrate forms a white precipitate of silver ferrocyanide, insoluble in dilute nitric acid or ammonium hydroxide, but soluble in alkali cyanide solutions. If, however, oxidised with concentrated nitric acid to silver ferricyanide, an orange coloured precipitate occurs, which is readily soluble in ammonium hydroxide.

Soluble copper salts form a characteristic chocolate brown precipitate of cupric ferrocyanide, when added to a ferrocyanide solution.

Soluble ferric salts produce, in neutral or acid solutions of ferrocyanides, Prussian blue. This forms the most delicate test for ferrocyanides, and is the most important reaction for the detection of ferrocyanides. In the case of insoluble ferrocyanides, they are heated with alkali hydroxide, when the metal hydroxide is precipitated and alkali ferrocyanide remains in solution. The precipitated metal hydroxide is separated from the solution by filtering. The filtrate is slightly acidified and added to a ferric chloride solution, when ferric ferrocyanide (Prussian blue) is immediately formed. Prussian blue is insoluble in dilute mineral acids, but dissolves in oxalic acid to a deep blue liquid

(formerly used as an ink) and in ammonium tartrate to a violet liquid. It is soluble in concentrated hydrochloric acid, but is again pecipitated on dilution with water. As a ferrocyanide salt it behaves like all other insoluble ferrocyanides and is decomposed on treatment with alkali hydroxides:

$$Fe_4(Fe(CN)_6)_3 + 12KOH = 4Fe(OH)_3 + 3K_4Fe(CN)_6.$$
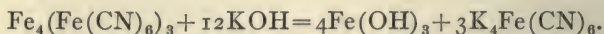
It is often necessary to detect cyanide salts in the presence of ferrocyanides. This can be easily undertaken as dilute hydrochloric acid evolves hydrocyanic acid from cyanides, while ferrocyanides are unaffected. To carry out the test the solid salts are placed in a small dish to which a small quantity of hydrochloric acid (dilute) is added, when the dish is at once covered with a watch glass or porcelain dish which is moistened with ammonium sulphide. If any cyanides are present, hydrocyanic acid will be evolved and be absorbed by the ammonium sulphide, forming ammonium thiocyanate. The cover is removed from the dish after allowing to stand for about 10 minutes. The ammonium sulphide on the cover is acidified with hydrochloric acid and a drop of ferric chloride solution added. The formation of a blood-red colouration determines the presence of cyanides.

Estimation of Ferrocyanides.

The estimation of ferrocyanides can be effected by precipitation, as silver, copper or iron salts, or by conversion into ferricyanide by oxidation with permanganate in acid solution. In applying this method, thicoyanates, sulphites, sulphides, thiosulphates, and other reducing agents must be absent. The process, which is well adapted for the assay of ferrocyanides in the absence of these impurities, is applied as follows: A quantity of material containing about 0.2 grm. of potassium ferrocyanide, is dissolved in water, the solution diluted to about 200 c.c., and placed in a white basin. The solution is acidified with sulphuric acid and assumes a bluish tint, it also becomes milky. The standard solution of potassium permanganate is run in till the yellow colour of the liquid changes to yellowish-red. The end reaction is tolerably definite. If a trace of ferric chloride be added to the liquid, the disappearance of the bluish-green colour will render the termination still more distinct. The reaction is:

$$5K_4Fe(CN)_6 + KMnO_4 + 4H_2SO_4 = 5K_3Fe(CN)_6 + 3K_2SO_4 +$$
$$MnSO_4 + 4H_2O.$$
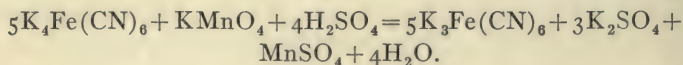
On account of the difficulty of obtaining the end point of the reaction, de Haen (*Ann. d. Chem. und Pharm.*, 1854, **90,** 160) states that it is preferable to set the permanganate by titrating a known quantity of pure potassium ferrocyanide, but the ordinary $N/10$ solution may be employed. Each c.c. $N/10$ permanganate used represents 0.04224 grm. crystallised, or 0.03683 grm. of anhydrous potassium ferrocyanide.

The impurities which are mostly found in commercial yellow prussiate are potassium sulphate, potassium carbonate, and potassium chloride. The sulphate can be determined by precipitating as barium sulphate from a slightly acidified solution. The carbonate can generally be estimated by titration with $N/10$ acid, using methyl orange as indicator. To estimate the potassium chloride, it is necessary to boil an aqueous solution of the ferrocyanide with mercuric oxide (free from chlorides) and to titrate the filtrate, after acidifying with nitric acid, with $N/10$ silver nitrate solution.

From salts of all other kinds than chlorides, bromides, iodides, iodates, cyanides, thiocyanates, ferricyanides, and sulphides, ferrocyanides may be separated by precipitating the liquid with silver nitrate in the presence of free nitric acid.

From chlorides and bromides (also thiocyanates) ferrocyanides may be separated by cupric sulphate in presence of free acid, and from these salts and from ferricyanides by precipitation with ferric sulphate.

In the filtrate from copper precipitate, after removing the excess of copper by hydrogen sulphide, the alkaline base of soluble ferrocyanides can be conveniently estimated.

Prussian blue and other insoluble ferrocyanides (the silver salt imperfectly) are converted into potassium ferrocyanide by boiling with solution of sodium hydroxide, the heavy metal being usually precipitated as oxide. If the metallic oxide be soluble in excess of alkali solution, it may be got rid of by passing carbon dioxide through the liquid, or in some cases magnesia may be substituted for the alkali hydroxide. In the filtrate, the ferrocyanide can be estimated by standard permanganate or other means.

The mother-liquors from ferrocyanide works may be assayed for ferrocyanide as follows: Remove any sulphide by boilng the liquid with lead carbonate; filter, acidify the filtrate with dilute sulphuric acid, and add from a burette a standard solution of cupric sulphate, containing 10 grm. of the crystallised salt to the litre. The addition is continued until a strip of filter paper, immersed so that the clear

liquid may rise by capillary attraction, gives no blue colour when touched with a drop of ferric chloride.   The precipitating power of the copper solution is ascertained by means of pure potassium ferrocyanide. About 0.2 grm. of the salt should be used, dissolved in 50 c.c. of water. Thiocyanates do not interfere with this method.

O. Knublauch (*J. Soc. Chem. Ind.*, 1889, **8**, 733) takes a convenient quantity of the ferrocyanide solution and adds to it slightly more copper sulphate solution than is required, as shown by the ferric chloride indicator applied as above; too little copper sulphate causing decomposition even with pure ferrocyanide.   The solution is next filtered, poured into hot ferric chloride solution (containing 60 grm. ferric chloride, and 200 c.c. hydrochloric acid, sp. gr. 1.19 per litre), and the whole filtered at about 80°.   The precipitate is washed somewhat with hot water, decomposed with a 10% solution of potassium hydroxide, and the filtrate titrated with standard cupric sulphate solution.   If it be thought desirable, the filtrate from the Prussian blue precipitate may be retreated in the same way, and the numbers obtined by titration added as a correction to the first result.

Instead of using cupric sulphate, a solution of ferric sulphate or chloride may be employed.   When this is added with vigorous agitation to a liquid containing a soluble ferrocyanide, a deep blue liquid results, which on further addition becomes turbid, and when exactly sufficient iron solution has been added for the reaction, $3K_4Fe(CN)_6 + 4FeCl_3 = 12KCl + Fe_4(Fe(CN)_6)_3$, the Prussian blue coagulates and the solution becomes perfectly clear.   The end reaction may also be observed as in the last process.   If thiocyanates be present, the least excess of iron solution will cause the liquid to assume a deep red colour. The change from blue to red furnishes a very definite end-reaction.

This process may be conveniently employed for the assay of ferrocyanide in the dye-vats and in "metal," which is the name given to the crude product in the manufacture of ferrocyanide.   In the case of alkaline liquids, the solution must be first acidified with dilute sulphuric or nitric acid.

Moldenhauer and Leybold (*J. f. Gasbel.*, 1889, **32**, 155) state that, when using Knublauch's method, the end of the titration was often uncertain.   They recommend the following procedure: 50 grm. of the substance containing ferrocyanide are placed in a flask with a 100 c.c. of a solution of 10% sodium hydroxide and 2% sodium carbonate, heat is applied to hasten the decomposition, and the whole then diluted

to 1030 c.c. 100 c.c. of the filtrate are evaporated down in a platinum or porcelain dish and treated with 25 c.c. of dilute sulphuric acid (10%). After further evaporation the excess of acid is driven off and the organic matter destroyed by ignition. The yellow residue consists of ferric sulphate and sodium sulphate, which is dissolved in dilute sulphuric acid. The iron is reduced to the ferrous state by means of zinc and titrated with permanganate solution. From the iron thus found the amount of Prussian blue is calculated. A blank experiment should be made to ascertain the amount of a slight correction for impurities in the zinc.

A very simple method of estimating small quantities of ferrocyanides present in soda-lyes has been described by Hurter (*Chem. News*, 1879, **39**, 25). These liquors contain sodium ferrocyanide, cyanate and thiocyanate, but of these the first only is objectionable, on account of the brownish colour it imparts to the finished product. Sodium cyanide may occasionally be present, in which case it may be converted into ferrocyanide by adding a small quantity of ferrous sulphate, boiling and filtering. The following are the details of Hurter's method: 100 c.c. of the strong soda-lye are boiled with solution of bleaching powder in quantity sufficient to convert all sulphides and thiosulphates into sulphates, and the ferrocyanide into ferricyanide. The liquid is then acidified and freed, as far as possible, from the excess of chlorine by warming and agitating it. It is then titrated with $N/20$ solution of cupric nitrate, prepared by dissolving 3.170 grm. metallic copper in as little nitric acid as possible, and diluting to 1,000 c.c. On adding this solution to the acidified solution containing ferricyanide, a yellow precipitate of cupric ferricyanide is formed. Drops of the thoroughly mixed liquid are taken up with a glass rod, and added to drops of 1% solution of crystallised ferrous sulphate on a porcelain plate. As long as insufficient copper solution has been added to combine with the whole of the ferricyanide present, the deep blue ferrous ferricyanide is formed on the porcelain. When the liquid no longer contains soluble ferricyanide, the indicator acts on the copper precipitate, and reduces it to the characteristic chocolate-coloured cupric ferrocyanide. Hence the end of the reaction is indicated by a brown colour being produced on the porcelain instead of the blue first obtained. Each c.c. of the copper solution added before this result is obtained represents 0.01013 grm. sodium ferrocyanide in the liquid. The method is not suitable for the determina-

tion of large quantities of ferrocyanide, as the colour of the copper precipitate obscures the blue colour, and the precipitate is not always of a definite composition. It is evident that this process is also adapted for the direct estimation of small quantities of ferricyanide, into which the ferrocyanide has first to be converted.

K. Zulkowsky (*Ding. Polyt. Jour.*, 1883, **249,** 168) recommends a process for the assay of ferrocyanide melt which is based on the reaction which occurs between potassium ferrocyanide and a soluble zinc salt. A standard solution of zinc sulphate is acidified with sulphuric acid, heated to boiling, and the ferrocyanide solution next run in from a burette. The point when ferric chloride gives a blue colouration to a strip of filter paper moistened with a drop of the mixed solutions shows the end reaction. Care must be taken that none of the zinc precipitate touches the ferric chloride on the filter paper.

In applying the above process R. Gasch (*J. f. Gasbel.*, 1889, **32,** 968) employs a 1% solution of uranium acetate, with which indicator ferrocyanides give a brown colouration. Gasch also uses a standard 2% solution of potassium ferrocyanide, against which he titrates the zinc solution, instead of using a standard solution of zinc sulphate as in the original process of Zulkowsky. For the determination of ferrocyanides in old gas waste, etc., Gasch rubs 20 grm. of the substance in a mortar with 15 to 20% sodium hydroxide, when warm water is added until the solution is of a thin consistency. It is next made up to a known volume, filtered and poured into a burette, and titrated as above, using uranium acetate solution as indicator. When the ferrocyanide is only present in very small amount, it is preferably precipitated as Prussian blue, filtered and dissolved in sodium hydroxide solution, when it is titrated as in the preceding case.

Analysis of Spent Purifying Mass of Gas Works.

Coal gas is purified from its cyanogen compounds, together with hydrogen sulphide by passing it over ferrous oxide or through an alkaline or neutral liquid containing ferrous hydroxide suspended, or else through solutions of ferrous salts. The affinity of cyanogen compounds for ferrous compounds is set forth on page 478. In the coal gas industry this spent mass, which is known under various names, as spent oxide, blue sludge, cyanide sludge or blue cake according to the state in which the spent mass is sold for manufacturing ferrocyanides, respectively cyanide salts, forms to-day a very important by-product.

This substance varies considerably in its contents of cyanogen which is its most valuable attribute. Analyses of this spent mass show a composition varying as follows:

Water,	16 to 30%
Sulphur,	25 to 55%
Prussian blue,	2 to 12%
Ammonium thiocyanate,	2 to 9%
Ammonia,	0.3 to 3%

For an important product as the spent mass, which varies so much in its composition, it is very important to have accurate analytical methods, not only to prevent dispute between seller and buyer, but also to be able to satisfactorily determine the yields in the purifying process. Unfortunately the analysis of the spent purifying mass, on account of the many complex compounds it contains, offers great difficulties to the chemist. A host of chemists have tried their hand at devising satisfactory methods and therefore a comparatively large number of methods have been worked out, but few have survived in commercial laboratories. Several, however, give useful figures in most of the cases, but it is customary for sellers and buyers to agree on a common method or else the name of the method used is generally given with the test.

The character of this work will not allow an exhaustive description and discussion of all the methods proposed, but the most reliable and the most employed methods are given below.

A very important part of the analysis consists in the proper sampling of the goods. Greatest care must be used in obtaining an accurate average sample and it is best to shake or stir (according to its condition) each container and then to take a proportional amount from each, *i.e.*, from a large container a proportionally larger amount than from a small container. The samples taken are well mixed and quartered down until about 1000 grm. is reached.

The moisture determination is liable to give erroneous results and can cause chemical decomposition to take place. It is customary to take 50 grm. spread out and heat in a drying oven at 70° for 4 hours.

The oldest method for the estimation of the cyanogen present in the spent gas purifying mass is due to Zulkowsky (see page 514). It was originally worked out for testing the ferrocyanide melt and was later used for testing the spent purifying mass of the gas works. Gasch (see page 514) improved on this method by using uranium acetate in-

stead of ferric chloride.   Moldenhauer and Leybold's method described on page 512 is also applicable for testing the spent mass.

De Koningh (*Zeit. f. angew. Chem.*, 1898, **11,** 463) takes the alkaline extract of the mass after filtering and evaporates it to dryness.   The residue is fused with saltpetre and soda and the melt dissolved in hydrochloric acid; the iron is then estimated gravimetrically by precipitation with ammonia.   Donath and Margosches (*Zeit. f. angew. Chem.*, 1899, **12,** 345) treat the filtered alkali extract with brominated sodium hydroxide, precipitating the iron which is filtered off and dried. The dry precipitate is dissolved in hydrochloric acid and the iron estimated volumetrically according to Moldenhauer and Leybold or gravimetrically according to de Koningh.

The above methods of estimation assume that all the iron in the filtrate is present as alkali ferrocyanide; this is not necessarily the case. A portion of the iron goes into solution in the form of other organic compounds, from which it is not precipitated by alkali sulphide. These facts are corroborated by Luehrig's investigations (*Chem. Ztg.*, 1902, **26,** 1039) who always obtained too high results by these method.

Knublauch (*J. f. Gasbel.*, 1889, **32,** 450) worked out a methods for the estimation of cyanogen in the spent purifying mass, suggested by Bohlig and founded on the technical process for working up the cyanide sludge.   The spent mass is extracted with alkali hydroxide and filtered, prussian blue is precipitated from the filtrate by means of a soluble ferric salt, the precipitate being again dissolved in alkali hydroxide and the formed alkali ferrocyanide titrated with copper sulphate. This method is extensively used and is given here in detail.

An average sample, as described on page 515, is placed on a clean plate or table, the lumps are broken up and the whole sample is well mixed up several times, finally spreading the whole out in a thin layer. It is now cut up into several squares, and from each a spoonful is taken so that the collected sample is about 1,000 grm.   This sample is placed in a well stoppered bottle and preserved for analysis.

A large weighed portion of this sample, 200 to 250 grm. is placed on a sieve, which is covered with paper, and carefully heated in a drying oven at 50 to 60° for about 6 hours.   When this sample is taken out of the oven it is allowed to stand in the air a few hours, until a constant weight is obtained.   The difference between this weight and the original is termed the "atmospheric moisture content."   This air dried mass is now finely powdered in an iron mortar, until it all passes through

a 200 mesh sieve; if any small pieces of wood remain on the sieve, which frequently occurs, they are cut up fine with a knife until they pass through the sieve. This mass is then placed in a glass stoppered bottle.

10 grm. of this air dried material are introduced into a 250 c.c. volumetric flask, to which is added 50 c.c. of a 10% potassium hydroxide solution, the mixture being allowed to stand for about 16 hours at room temperature, with frequent stirring during the first and last 2 hours. It has been suggested with advantage, to place the 10 grm. of material in a porcelain mortar pour onto it 50 c.c. of the 10% potassium hydroxide and frequently mix the mass carefully with the pestle. This latter method prevents the possibility of lumps forming and furnishes a much better contact for the reacting substances. After 16 hours the mixture in the porcelain mortar is washed into a 250 c.c. volumetric flask and filled up to the mark with water, adding then 5 c.c. more water to correspond to the amount of solid substance present in the flask. The mass is well shaken up and then filtered. The potassium ferrocyanide cannot be directly estimated in this filtrate as several foreign substances present would interfere with the titration, and the potassium ferrocyanide must be separated from the solution. For this purpose an iron chloride solution is prepared by dissolving 60 grm. ferric chloride in water, adding 200 c.c. hydrochloric acid and making the whole up to 1,000 c.c. 25 c.c. of this solution is placed in a beaker while 100 c.c. of the alkaline filtrate is gradually added, constantly stirring and heating during this period. The precipitated Prussian blue is allowed to settle and then filtered through a folded filter, which is kept covered, and the solution is kept warm while filtering. The precipitate is washed twice with hot water and is then carefully placed, together with the filter paper, in a beaker to which 20 c.c. of a 10% potassium hydroxide is added. This reaction can be hastened by heating the beaker, but great care must be exercised to prevent any local overheating which would decompose the Prussian blue. The resulting mass is washed into a 250 c.c. volumetric flask, the flask being then filled to the mark. It is customary to add still about 1 grm. freshly precipitated lead carbonate to precipitate any soluble sulphide which might be present. The flask is now properly shaken and the contents filtered through a dried folded filter into a flask. The ferrocyanide is estimated in the acidified filtrate by titration with copper sulphate, using ferric chloride as indicator for the drop test reaction.

The copper sulphate is prepared by dissolving 12 to 13 grm. pure copper sulphate in 1,000 c.c. water and standardizing same against a solution of 4 grm. pure potassium ferrocyanide in 1,000 c.c. water. 50 c.c. of this potassium ferrocyanide solution are pipetted into a beaker and acidified with 5 c.c. sulphuric acid (20%). Now copper sulphate solution is allowed to run in, while constantly stirring, until by adding a drop of the solution to ferric chloride no more Prussian blue is detected. For the drop test reactions, a drop of the solution is taken by means of the glass rod and placed on a heavy piece of iron free filter paper (drop reaction paper) and allowed to spread, when a drop of ferric chloride solution (1%) is placed near the same, in such a manner that only the edges of the spreaded drops, flow into each other. Copper ferrocyanide and also the zinc salt is converted by ferric chloride into Prussian blue, so that a mixture of these two will always give erroneous results. Toward the end of the titration it is necessary to wait a definite time (2 minutes) for the drop test reaction to take place, as it only takes place very slowly. The end point of the titration can be intensified by taking 1 c.c. of the solution, filtering through a small filter into a test-tube containing a drop of ferric chloride solution, mix and observing by transmitted light against a white screen. If there is no indication of the blueing after 1/2 minute the titration is considered finished. This filter test reaction always gives higher results than the drop test reaction, so that the same methods of estimating the end point of the titration must be used in standardizing the copper sulphate solution as in testing the solution from the spent mass. It is very convenient to use the drop test reaction for the first titration and to follow up with several titrations using the filter test reaction, and takin · the average of the four last esitmations. The reaction of this titration takes place in two phases:

$$(1)\ K_4Fe(CN)_6 + CuSO_4 = K_2CuFe(CN)_6 + K_2SO_4.$$

$$(2)\ K_4CuFe(CN)_6 + CuSO_4 = Cu_2Fe(CN)_6 + K_2SO_4.$$
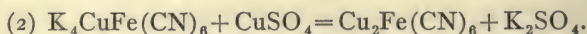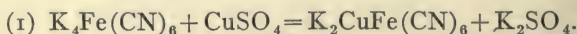
The results are generally expressed in per cent. cyanogen present, also occasionally in per cent. of Prussian blue, $Fe_7(CN)_{18}$. The method requires some practise to always attain the true end point of the reaction.

Drehschmidt (*J. f. Gasbel.*, 1892, **35**, 221) made an investigation of the Knublauch method and claims that some of the foreign materials,

which would interfere with the titration, are carried along when the potassium ferrocyanide is separated from the solution. Drehschmidt therefore suggests that the cyanogen contents be estimated direct, and he has modified the Rose-Finkener method for this purpose. According to Drehschmidt's method, the spent purifying mass containing the cyanogen compounds is boiled with mercuric oxide, forming mercuric cyanide. Some ammonium sulphate is added to prevent any fixed alkali interfering with the reaction. Besides mercuric cyanide insoluble mercuric thiocyanate is also formed. In cases where thiocyanate is present in any abundance it is customary to add a little mercuric nitrate to be sure that all the thiocyanate is converted into insoluble mercuric thiocyanate. The mercuric cyanide is decomposed by reducing with zinc dust, free from chlorine, in a strong ammoniacal solution to ammonium cyanide. Alkali hydroxide is added to prevent the possibility of hydrocyanic acid volatilising and the cyanogen is estimated according to Volhard's volumetric method.

In carrying out this method Drehschmidt proceeds as follows: 10 grm. of the average sample of the air dried material, prepared as described on page 515, are introduced into a 500 c.c. volumetric flask. To this 150 c.c. water, 1 grm. ammonium sulphate and 15 grm. mercuric oxide are added; the mixture is allowed to boil for 15 minutes, the reaction having been completed in this time. The flask is cooled, then 1/2 to 1 c.c. of a saturated mercuric nitrate solution is added while stirring and also enough ammonium hydroxide till no more precipitation occurs, when the flask is filled to the mark and an additional 8 c.c. of water is introduced to compensate the volume of the precipitate formed. The contents of the flask are well shaken and then filtered through a dry folded filter. 200 c.c. of the filtrate, corresponding to 4 grm. of the original air dried mass, are placed in a 400 c.c. volumetric flask, to this is added 6 c.c. ammonium hydroxide (0.91 sp. gr.) and 7 grm. zinc dust (free from chlorine) then stirred for some time. Now 2 c.c. of potassium hydroxide (30%) is added before filling flask up to the mark. An extra 1 c.c. of water is added, the contents mixed and again filtered through a dry folded filter. 100 c.c. of the filtrate, corresponding to 1 grm. of the original air-dried mass is introduced into a 400 c.c. volumetric flask which contains 35 c.c. of $N/10$ silver nitrate solution and 25 c.c. dilute nitric acid (10%). The flask is shaken, which tends to gather the precipitate, and then filled up to the mark and filtered through a dry folded filter. 200 c.c. of the

filtrate are placed in a beaker and the excess of silver is estimated according to Volhard by titration with $N/10$ ammonium thiocyanate, using a saturated iron ammonium alum solution as indicator. The number of c.c. of this solution used is subtracted from the amount of $N/10$ silver nitrate added, and the difference gives the amount of silver required for 1 grm. air dried mass.

$$1 \text{ c.c. } N/10 \text{ silver nitrate} = 0.2598\% \text{ CN}$$
$$= 0.7042\% \text{ K}_4\text{Fe(CN)}_6$$
$$= 0.4782\% \text{ Fe}_7\text{(CN)}_{18}$$

Burschell (*J. f. Gasbel.*, 1893, **36,** 7) and Lubberger (*J. f. Gasbel.*, 1898, **41,** 124) do not consider Drehschmidt's method reliable and suggest a combination of Knublauch and Drehschmidt's methods, namely, by extracting and separating as in Knublauch's method, but using less time for the extraction, and then estimating the cyanogen content according to Drehschmidt.

It is not clear why Burschell desires to so complicate the process of estimation, because the most reliable part of Knublauch's method is the titration with copper sulphate.

Nauss (*J. f. Gasbel.*, 1900, **43,** 696) worked out a method to estimate the ferrocyanogen alkalimetrically. He takes the precipitated Prussian blue of Knublauch, digests with a known quantity of sodium hydroxide and titrates the excess with acid. 10 grm. of the average air dried sample are placed in a flask with 50 c.c. sodium hydroxide solution (10%) for 15 hours at room temperature. The flask is now filled to the mark and 5 c.c. water are added in excess, shaken and filtered through a dried filter. 50 c.c. of the filtrate are let into 10 to 15 c.c. of an acid iron alum (200 grm. iron alum and 100 grm. sulphuric acid per litre). The mixture is heated for a short time when the formed Prussian blue is filtered off and washed. The precipitate, together with the filter, is placed in a flask with some water and brought to a boil, when enough $N/50$ sodium hydroxide is added till all the Prussian blue is decomposed and the excess of sodium hydroxide is titrated in the hot solution with $N/50$ acid. The end of the reaction is detected by the observance of a yellowish-green colour, which is the re-formation of Prussian blue. This method may be used when no great accuracy of results is necessary, but, owing to the fact that the Prussian blue precipitate cannot be washed out so well and that no time is saved by the method, it does not offer any advantages over the other methods.

There is still another method for the estimation of the total ferro-cyanogen in the spent purifying mass, which is described by Feld (*J. f. Gasbel.*, 1903, **46**, 642). 2 grm. of the air dried spent mass are placed in a glass mortar to which 1 c.c. 3N magnesium chloride and 2 c.c. water are added. The mixture is triturated till it becomes as fine as possible when it is evaporated to dryness on a water-bath. After cooling the mass is ground with 5 c.c. of an 8N sodium hydroxide solution until all is decomposed, which generally requires about 5 minutes. 10 c.c. of a 3N magnesium chloride solution is added to the paste while stirring, and the whole is washed with hot water through a funnel into a distilling flask. Now 20 c.c. of the magnesium chloride solution is added and enough water until a volume of about 150 to 200 c.c. is attained, when the whole is heated to boiling. Now 100 c.c. of $N/10$ mercuric chloride solution is added to the boiling liquid and the boiling is continued for 5 or 10 minutes longer, then 30 c.c. of 4N sulphuric acid is added and the liberated hydrocyanic acid is distilled over. The hydrocyanic acid is caught in 20 c.c. 2N sodium hydroxide, and after adding 5 c.c. of $N/4$ potassium iodide solution the same is titrated with $N/10$ silver nitrate solution. If the distillate is cloudy, it is poured into a volumetric flask, 0.5 grm. lead carbonate added, filled to the mark, mixed and filtered through a dry filter. Half of the filtrate is taken and titrated with silver nitrate solution.

Witzeck (*J. f. Gasbel.*, 1904, **47**, 545) made a thorough investiga-tion of this method and describes the following apparatus for carrying out the distillation. A round bottom flask is provided with a double bored rubber stopper which contains a separatory funnel and distilling column. The latter is connected with a condenser which dips into an Erlenmeyer flask also provided with a double bored rubber stopper, the second hole is connected with a three bulb tube containing sodium hydroxide.

For the estimation of the *total cyanogen* in the spent purifying mass 2 grm. of the same are triturated in a glass mortar with 1 c.c. iron sulphate (278 grm. per 1,000 c.c.) and 5 c.c. sodium hydroxide (320 grm. per 1,000 c.c.) for 5 minutes. While stirring, 30 c.c. magnesium chloride solution (610 grm. per 1,000 c.c.) are added and the whole is washed with hot water into the distilling flask until the volume is about 200 c.c. Now the analysis takes the same course as described above. The whole operation requires about 1 1/2 hours and gives

results slightly higher than by Knublauch-Drehschmidt's method. On account of its simplicity and rapidity, this method deserves more attention than it has up to the present received.

A method described by H. E. Williams (*J. Soc. Chem. Ind.*, 1912, **31,** 468) is stated to give equally as good results as the Feld method described above but with fewer operations.   The best means of carrying out the estimation according to this method is to dissolve 0.5 grm. of the ferrocyanide in 100 c.c. water and transfer to a 250–300 c.c. distilling flask and then add 0.05–0.1 grm. of cuprous chloride, dissolved in a few drops of hydrochloric acid, or a saturated solution of sodium or potassium chloride; gently shake the flask to mix the constituents and then add 25–30 c.c. of N/4 sulphuric acid.   The mixture is then distilled through a condenser into absorption flasks containing sodium or potassium hydroxide.   The ferrocyanide is completely decomposed, and the hydrocyanic acid distilled off in about half an hour's gentle boiling.   The alkaline liquor is washed out of the absorption flask and condenser with distilled water and the cyanide solution estimated in the usual manner with N/10 silver nitrate solution.   On account of the large amount of alkali present a few drops of a 10% potassium iodide solution should be added.

The theory of this reaction is still under investigation but it appears that the cuprous chloride decomposes the ferrocyanide, forming cuprous cyanide, which is then decomposed by the sulphuric and hydrochloric acids present into hydrocyanic acid and cuprous chloride. The regenerated cuprous chloride attacks a further quantity of ferrocyanide and sets a cycle of reactions in motion, until all the ferrocyanide has been decomposed.

In commerce only the cyanogen or Prussian blue contents of the spent purifying mass are of value, but in many factories it is customary also to estimate the sulphur, ammonia and thiocyanate contents.

For the estimation of the elementary *sulphur* the air dried spent mass is treated with carbon bisulphide in a Soxhlet extraction apparatus.   15 grm. of the mass in placed in an extraction thimble and covered with some degreased cotton wool or asbestos.   The extraction thimble is then placed in the Soxhlet extracting apparatus.   This extraction apparatus is then set on a weighed round bottom flask (about 200 c.c. capacity) which contains 100 c.c. freshly distilled carbon disulphide, and connected above with the usual reflux condenser.   The extraction lasts until the carbon bisulphide flowing from the extraction

apparatus remains colourless. It generally requires about 20 extractions. Care should be taken not to allow the sun to shine on the apparatus as it tends to decompose the carbon disulphide producing sulphur. After the extraction is completed the Soxhlet apparatus is removed and an ordinary condenser attached to the flask, when the carbon disulphide is distilled over. The last traces of carbon disulphide vapours are removed by passing air through the apparatus and then the flask is weighed with the sulphur. The increase of weight found gives the elementary sulphur contents.

The sulphur generally contains a little tar and should be freed from this impurity. It is general only to wash the sulphur a few times with ether and then heat the flask on the water bath. If, however, it is necessary to obtain accurate results, a portion of the sulphur must be taken, oxidised with fuming nitric acid and the sulphuric acid precipitated as barium sulphate.

Ammonia is present both in soluble and insoluble form. To determine the *soluble ammonia* 25 grm. of the mass are digested in a 500 c.c. volumetric flask with water for a whole day, the flask is then filled to the mark, shaken and filtered through a dry folded filter. 200 c.c. of the filtrate, corresponding to 10 grm. of the mass, are distilled with milk of lime. The distillate is received in a known quantity of $N/10$ acid and the excess acid titrated with $N/10$ alkali hydroxide.

To estimate the *total ammonia* contents, 10 grm. of the mass is distilled direct with milk of lime and water, and the distillate is treated as above. The difference between the total ammonia and soluble ammonia gives the contents of insoluble ammonia.

The thiocyanates in the spent purifying mass are also present as soluble and insoluble compounds, but it is generally only necessary to estimate the soluble thiocyanates (*i.e.*, ammonium thiocyanate). 50 grm. of the mass are digested with 500 c.c. water in a 1,000 c.c. volumetric flask for a day at room temperature. The flask is now filled up to the mark and 30 c.c. water added in excess, to correct the volume taken up by the residue. The contents of the flask are well shaken and then filtered through a dry folded filter. For the estimation of thiocyanates in the filtrate see under thiocyanates (page 550).

The estimation of sulphates, ferrous oxide, ferric oxide, etc., in the spent purifying mass offers no unusual obstacles and the ordinary usual methods can be adapted for the same.

Ferricyanides.

Ferricyanides may be regarded as compound salts of ferric cyanogen $(Fe(CN)_3)$ with the cyanide of some other metal. They are obtained by the action of an oxidising agent on ferrocyanides.

Hydroferricyanic Acid, $H_3Fe(CN)_6$, is prepared by treating a cold saturated potassium ferricyanide with two to three times its volume of concentrated hydrochloric acid. The acid crystallises in brownish-green needles, which are soluble in water and alcohol, insoluble in ether and turn blue when exposed to the air.

Potassium Ferricyanide, $K_3Fe(CN)_6$, also known as red prussiate, can be prepared by oxidising with any of those reagents which oxidise ferrous oxide to ferric oxide. To manufacture potassium ferricyanide chlorine gas is passed through a solution of potassium ferrocyanide until a sample shows no blue precipitate with ferric chloride solution. The solution is then made weakly alkaline and evaporated for crystallisation. The oxidising reaction is as follows:

$$2K_4Fe(CN)_6 + Cl_2 = 2K_3Fe(CN)_6 + 2KCl.$$
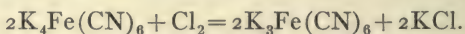
Walker (*J. Amer. Chem. Soc.*, 1895, **17**, 68) operates as follows: 26 parts of potassium ferrocyanide are dissolved in 200 c.c. of cold water, and 8 parts of strong hydrochloric acid added. 2 parts of potassium permanganate in 300 parts of water are then introduced slowly. The solution should give a brown colour, but no precipitate with ferric chloride solution. The excess of acid is neutralised with calcium or barium carbonate, and the solution evaporated on the water-bath. The first crystals will be pure, while the subsequent crops of crystals may contain chlorides, which can be eliminated by fractional crystallisation.

A process for the manufacture of potassium ferricyanide has been described by G. Kassner (*Chem. Ztg.*, 1889, **13**, 1701). It consists of adding calcium plumbate to a solution of potassium ferrocyanide and passing a stream of carbonic acid gas when the reaction proceeds according to the equation:

$$2K_4Fe(CN)_6 + PbO_2(CaO)_2 + 4CO_2 = 2K_3Fe(CN)_6 + PbCO_3 + 2CaCO_3 + K_2CO_3.$$
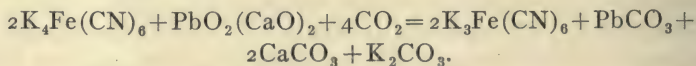
The carbonates of lead and calcium separate as insoluble precipitate which can be filtered off and regenerated by a simple roasting, while

the solution contains potassium ferricyanide in a pure form and potassium carbonate as valuable by-product. The calcium plumbate is prepared by roasting oxide or carbonate of lead with calcium carbonate at a low red heat. In the conversion of potassium ferrocyanide into ferricyanide by means of lead peroxide a quantity of potassium hydroxide is set free which must be neutralised to prevent the reformation of potassium ferrocyanide, and carbonic acid suffices for the neutralisation.

Potassium ferricyanide (also called Gmelin's salt) is known in commerce as "red prussiate of potash" (German, Rothesblutlaugensalz; French, Prussiate rouge de potasse); it crystallises in ruby red rhombic prisms, which are anhydrous. Potassium ferricyanide has an aperient action, but is not poisonous. Potassium ferricyanide has a sp. gr. of 1.845. According to Wallace (*Ding. Polyt. Jour.*, 1856, **142**, 52) 100 parts of water dissolve:

Temperature.	Parts $K_3Fe(CN)_6$.
4.4	33
10.0	36
15.5	40.8
37.8	58.8
100.0	77.5
104.4	82.6

The solutions according to Schiff (*Ann. Chem. Phys.*, **113**, 200) have the following sp. gr. at 15°:

% $K_3Fe(CN)_6$	Sp. gr.	% $K_3Fe(CN)_6$	Sp. gr.
1	1.0051	16	1.0891
2	1.0103	17	1.0951
3	1.0155	18	1.1014
4	1.0208	19	1.1076
5	1.0261	20	1.1139
6	1.0315	21	1.1202
7	1.0370	22	1.1266
8	1.0426	23	1.1331
9	1.0482	24	1.1396
10	1.0538	25	1.1462
11	1.0595	26	1.1529
12	1.0653	27	1.1596
13	1.0712	28	1.1664
14	1.0771	29	1.1732
15	1.0831	30	1.1802

The salt is only slightly soluble in aqueous alcohol and insoluble in absolute alcohol. The solution has a strong yellow colour. By exposure to light, or evaporation to dryness, potassium ferricyanide is partially decomposed with formation of ferrocyanide. This change is hastened by the presence of potassium oxalate, mercuric chloride, etc. Ammonium ferricyanide is reduced by light more readily than the potassium salt. Paper wetted with mixed solutions of a ferricyanide and ferric chloride becomes blue on exposure to light, a fact utilised in photography to obtain the so-called "ferro-type" prints.

Potassium ferricyanide treated with chlorine forms Prussian green, $3Fe(CN)_2.10Fe(CN)_3$. Reducing agents easily convert potassium ferricyanide to potassium ferrocyanide. Bloxam (*Chem. News*, 1883, **48**, 73) finds that by boiling with a little potassium cyanide the following takes place:

$$2K_3Fe(CN)_6 + 2KCN + 2H_2O = 2K_4Fe(CN)_6 + HCN + NH_3 + CO_2.$$
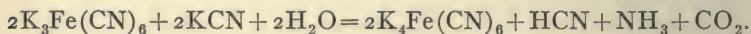
Similar to potassium ferrocyanide, potassium ferricyanide forms a large number of double salts with nitrates and haloids. It also forms mixed double cyanides with the alkali and earth alkali metals, etc.

Turnbull's Blue is ferrous ferricyanide, $Fe_3(Fe(CN)_6)_2$, and is prepared by precipitation from a ferrous salt solution with potassium ferricyanide. A dark blue precipitate is formed which has similar behaviour and chemical properties to Prussian blue. If an excess of the iron cyanogen salt is used, when either Prussian or Turnbull's blue are precipitated, a precipitate containing alkali is obtained, which is soluble in pure water, and can be salted out of its solution.

Guignet (*Compt. Rend.*, 1889, **108**, 178) prepares this soluble blue double cyanide as follows: 110 grm. potassium ferricyanide are dissolved in water, and when boiling intermittently decomposed with 70 grm. crystallised iron sulphate, and then boiled for 2 hours. The precipitate is filtered off and washed with water until the water comes through the filter coloured strongly blue. The precipitate is now dried at 100°. Soluble Prussian blue can also be prepared by mixing pasty Prussian blue with oxalic acid solution for a long period and then separating the oxalic acid with alcohol. Matuchek (*Chem. Ztg.*, 1902, **26**, 92) prepared a soluble Prussian blue by treating potassium ferricyanide with oxalic acid, which was soluble in absolute alcohol. The formula of soluble Prussian blue, dried at 100°, is $KFe.Fe(CN)_6 + 7/4 H_2O$.

Detection of Ferricyanides.

Neither the iron nor the cyanogen of ferricyanides can be recognised by the ordinary tests, and the salts as a class are not violently poisonous. The ferricyanides of the light metals have a red colour, and are soluble in water. The ferricyanides of the heavy metals are mostly insoluble.

By the action of heat and strong acids, the ferricyanides are decomposed in a very similar manner to the ferrocyanides (page 509).

An aqueous solution of potassium ferricyanide gives the following reactions:

Hydrogen sulphide readily reduces a ferricyanide with formation of a ferrocyanide.

In the presence of alkali hydroxide, stannous, manganous, ferrous, plumbous and chromic oxides reduce ferricyanides to ferrocyanides. Alcohol, oxalates, cyanides, sulphites and phosphites also exert a reducing action, and indigo is bleached. Sugar, starch and cellulose likewise reduce ferricyanide.

Hydrogen peroxide is decomposed by alkaline ferricyanide, with evolution of oxygen.

When boiled with yellow mercuric oxide and water, ferricyanides are completely decomposed with formation of mercuric cyanide and precipitation of oxide of iron.

Silver nitrate precipitates orange-red silver ferricyanide, $Ag_3Fe(CN)_6$, insoluble in dilute nitric acid, but soluble in ammonia.

Ferrous sulphate produces a deep blue precipitate (Turnbull's blue) of ferrous ferricyanide, $Fe_3(Fe(CN)_6)_2$, insoluble in dilute acids, but decomposed by hot alkali hydroxides with formation of soluble ferrocyanide and black ferroso-ferric oxide. This reaction distinguishes it from Prussian blue, which in appearance it closely resembles, but which yields yellow-brown ferric hydroxide, without any ferrous oxide, on boiling with alkali hydroxides.

Ferric chloride, if free from ferrous salt, produces merely a brownish colouration in solutions of ferricyanides free from ferrocyanides.

When treated with a nitrite and acetic acid, ferricyanides are converted into nitroprussides (see page 529).

Dilute sulphuric acid does not evolve hydrocyanic acid in the cold but only when heated, which distinguishes it from cyanides, which evolve hydrocyanic acid both in the cold and on heating.

On account of the great ease with which hydroferricyanic acid is

reduced, it is often difficult to detect the presence of the same, in fact it is sometimes impossible to detect it, especially is this liable to be the case with insoluble ferricyanide salts. By decomposing Turnbull's blue with alkali hydroxide, a mixture of ferrous and ferric oxide are obtained, while alkali-ferrocyanide is in solution, this is due to the alkali ferricyanide formed having oxidized part of the ferrous oxide to ferric oxide.

Estimation of Ferricyanides.

On account of the character of these compounds the methods of estimation depend on reduction.

Liesching (*Jahresber.*, 1853, 681) titrates potassium ferricyanide direct with an empirically standardised solution of arsenious sulphide in sodium sulphide, using cochineal as indicator. As soon as the cochineal is not discoloured by the drop test reaction, the reduction is completed.

In a concentrated hydrochloric acid solution potassium ferricyanide is reduced by potassium iodide, liberating free iodine:

$$K_3Fe(CN)_6 + KI = K_4Fe(CN)_6 + I$$
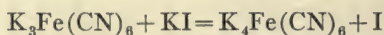
The liberated iodine can be titrated with sodium thiosulphate. Lenssen (*Ann. Chem. Pharm.*, 1854, **91,** 240) has based on this a method for ferricyanides. The liberated iodine has a tendency to oxidize part of the formed ferrocyanide back to ferricyanide, so that Mohr (Titrier-methoden, 1877, 284), modified this method by adding zinc sulphate to the solution and precipitating the ferrocyanide formed as zinc ferrocyanide, which is not acted on by the iodine. The method is to mix the dilute solution of the ferricyanide with potassium iodide and hydrochloric acid in excess, add an excess of solution of iron-free zinc sulphate, neutralise the free acid with a slight excess of sodium hydrogen carbonate, and estimate the liberated iodine by standard sodium thiosulphate and starch. The reaction is as follows:

$$2K_3Fe(CN)_6 + 4ZnSO_4 + 2KI + 2Na_2S_2O_3 = 4K_2SO_4 + 2Zn_2Fe(CN)_6 + 2NaI + Na_2S_4O_6$$
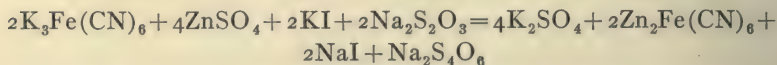
Each c.c. of $N/10$ thiosulphate required to react on the liberated iodine represents 0.0329 grm. of potassium ferricyanide.

Mohr (*Titriermethoden*, 1896, 249) reduces the potassium ferricyanide in alkaline solution with ferrous sulphate and titrates the formed potassium ferrocyanide. The potassium ferricyanide solution is made strongly alkaline, heated to boiling when a concentrated solution of ferrous sulphate is added. At first yellow ferric hydroxide is precipitated, by further addition of the ferrous sulphate ferrous hydroxide is also precipitated, which forms black ferroso-ferric hydroxide with the ferric hydroxide when boiled. As this precipitate only settles with difficulty, it is best to dilute the whole to 300 c.c., and filter two samples of 100 c.c. each, acidify the filtrate and titrate with $N/10$ permanganate solution. A sign, that the reduction of the potassium ferricyanide is complete, is the colourless filtrate and the formation of a black precipitate.

Quincke (*Zeit. f. anal. Chem.*, 1892, **31**, 1) has shown that the action of alkali hydroxide and hydrogen peroxide on potassium ferricyanide proceeds quantitatively according to the equation:

$$2K_3Fe(CN)_6 + 2KOH + H_2O_2 = 2K_4Fe(CN)_6 + 2H_2O + O_2$$
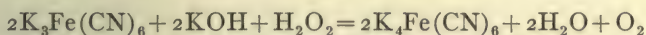
The volume of oxygen evolved is therefore a measure of the ferricyanide present.

The best mode of operating is to introduce 5 or 10 c.c. of the solution of the ferricyanide into the closed tube of a nitrometer filled with mercury, and then run in an equal measure of sodium hydroxide solution through the tap. This is followed by a solution of hydrogen peroxide, and the contents of the nitrometer are mixed by agitation. More hydrogen peroxide is then added to ensure the completion of the reaction, when a measure of water, equal to the combined aqueous liquids, is poured into the open limb, the level of the mercury in the two limbs adjusted, and the volume of gas read off. 1 c.c. of oxygen at 0° and 760 mm. = 0.02945 grm. potassium ferricyanide, or 0.0279 grm. at the ordinary temperature and pressure. Barium peroxide or any other earth alkali or alkali peroxide dissolved in hydrochloric acid may be substituted for hydrogen peroxide, provided that an excess of strongly alkaline ferricyanide be employed.

G. Kassner (*Archiv. der Pharm.*, 1890, **228**, 182) proposes to determine the ferrocyanide formed in the above method, and so obtain an estimate of the amount of ferricyanide originally present. After acidifying the cooled liquid with dilute sulphuric acid, and boiling off the excess of hydrogen peroxide, it is titrated with standard perman-

ganate solution.   Kassner also recommends the reaction as a conven-
ient means of preparing pure oxygen.

Feld's method (see page 52) can be used for the estimation of ferri-
cyanides in the same way as it is used for estimating ferrocyanides.

Nitroprussides or Nitroso-ferricyanides.

When a ferrocyanide is heated with nitric acid or when a ferricyanide
is treated with nitrous acid, a substitution product is formed in which
one of the cyanogen groups is replaced by nitrosyl.

Nitroprussic Acid, $H_2Fe(CN)_5NO$, is formed by treating potassium
ferro- or ferricyanide with nitric acid or the action of nitrous oxide
on hydroferrocyanic acid.   Nitroprussic acid is prepared by treating
barium nitroprusside with sulphuric acid or silver nitroprusside with
hydrochloric acid.

Nitroprussic acid forms dark red, deliquescent crystals, which are
easily soluble in water, alcohol and ether, but decompose by boiling
with water.   Of its salts sodium nitroprusside $Na_2Fe(CN)_5NO +$
$2H_2O$ is the most important and best known.   According to Overbeck
(*Jahresber.*, 1852, 438) it is prepared as follows: 4 parts of pulverised
potassium ferrocyanide are heated with 5 1/2 parts of nitric acid and
5 1/2 parts of water until a sample of the solution does not form any
blue precipitate with ferrous sulphate solution.   After allowing to cool
it is poured off from the potassium nitrate crystals and the mother
liquor is evaporated until no more potassium nitrate crystallises out.
The liquor is then neutralised with sodium carbonate and the sodium
nitroprusside is allowed to crystallise.

Hoffmann (*Zeit. f. anorg. Chem.*, 1889, **10**, 262) allows a concentrated
aqueous solution of ferrous sulphate to react with equal portions of
potassium cyanide and sodium nitrite.   A lively action takes place
whereby nitrogen oxide and nitrogen are evolved, while ferric hydrox-
ide is formed.   The mixture is allowed to stand for five hours at
ordinary temperature, when it is made weakly alkaline with sodium
hydroxide, heated for a short period to 25° and filtered.   The filtrate
is then evaporated for crystallisation.

Sodium nitroprusside, $Na_2Fe(CN)_5NO + 2H_2O$, crystallises in
monoclinic prisms.   It crystallises more readily than the potassium salt.
It forms deep red crystals very soluble in water, and is decomposed
on exposure to light with formation of a blue precipitate.   Sodium

nitroprusside was found to be twenty times more sensitive to light than potassium ferricyanide, and in presence of ferric chloride the decompostiton was still more rapid. The crystals dissolve in 2.5 parts of water at 16°. Etard and Bemont (*Compt. Rend.*, 1885, **99**, 1024) state that when the crystals are heated *in vacuo* at 440° they decompose:

$$Na_2Fe(CN)_5NO + 2H_2O = Na_2Fe(CN)_4 + NO + CN + 2H_2O.$$
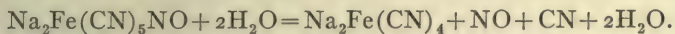
By carefully heating sodium nitroprusside in carbon dioxide atmosphere it forms Prussian blue, sodium ferrocyanide, cyanogen and nitrogen.

Soluble nitroprussides are unchanged by ferric salts. With silver nitrate they yield a flesh-coloured precipitate, insoluble in nitric acid, and salmon-coloured precipitates with ferrous and zinc salts. The last reaction distinguishes nitroprussides from cyanides of the formula $M_2Fe(CN)_6$ ("perferricyanides"), which give a green precipitate with zinc sulphate.

Hydrogen sulphide decomposes nitroprussides with formation of a ferrocyanide, separation of Prussian blue and sulphur, and production of iron nitrosulphide.

The chief interest attaching to the nitroprussides is the beautiful but transient violet colour which they yield with soluble sulphides. This reaction forms a delicate test both for sulphides and for nitroprussides. If one inch of hair be fused with sodium carbonate and the product dissolved in water, the presence of sulphur is recognisable by subsequently adding a nitroprusside. By operating in alcohol solution the colouring matter separates as a purple-blue oily compound, which gives a green powder when dried in vacuo. It readily decomposes into a sulphide and ferricyanide.

Sodium nitroprusside is also applied as a test for creatinine, which in presence of alkali hydroxide gives a fine ruby-red colouration, changing in a few minutes to a straw-yellow. Acetone also gives a red colour with alkaline nitroprusside solution, and other ketones and aldehydes give reactions ranging from yellowish-red to violet (Bitto, *J. Soc. Chem. Ind.* 1892, **11**, 847).

When a solution of a nitroprusside is rendered alkaline by sodium hydroxide it acquires a red tint, changing to orange, and on boiling the liquid the nitrosyl group exerts a reducing action, ferrous hydroxide being precipitated, nitrogen evolved, and sodium ferrocyanide and nitrite formed.

Carbonyl-ferrocyanides.

One of the cyanogen groups, in the ferrocyanogen ion, can be replaced by carbonyl, forming compounds of carbonyl ferrocyanides, which were discovered by Muller in 1888 (*Compt. Rend.*, 1887, **104,** 992).

Hydrocarbonyl-ferrocyanic acid, $H_3Fe(CN)_5CO$, is obtained by treating the copper salt with hydrogen sulphide and evaporating the aqueous solution in a desiccator over sulphuric acid. It forms laminated colourless crystals of astringent taste and is a strong tribasic acid. When the aqueous solution is boiled it forms hydrocyanic acid and a blue violet precipitate.

Potassium carbonyl-ferrocyanide can be produced by heating potassium ferrocyanide with carbon monoxide in a tube (Muller, *Compt. Rend.*, 1898, **126,** 1421.)

$$K_4Fe(CN)_6 + CO + 2H_2O = K_3Fe(CN)_5CO + NH_3 + KCOOH.$$
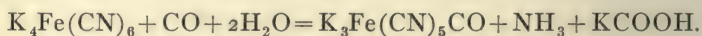
Potassium carbonyl-ferrocyanide crystallises in the monoclinic system and is very soluble in water.

The yellow-green copper carbonyl-ferricyanide, $Cu_3(Fe(CN)_5CO)_2$, and the blue-violet ferric salt, $FeFe(CN)_5CO$, are occasionally found in the products of the destructive distillation of coal, (Schutzenberg, *Compt. Rend.*, 1887, **104,** 992), and both are used for distinguishing the carbonyl-ferricyanide compounds from the ferrocyanide compounds.

Cobalticyanides.

This very permanent class of double cyanides is chiefly of interest from its application to the separation of nickel and cobalt. When potassium cyanide is added to a solution of cobalt, brownish-white cobaltous cyanide, $Co(CN)_2$, is formed; this dissolves in excess of potassium cyanide to form the easily decomposable double cyanide, $K_2Co(CN)_6$. With excess of potassium cyanide, red potassium cobaltocyanide, $K_4Co(CN)_6$, is formed. On heating the solution containing this, it is quickly converted, with evolution of hydrogen, into colourless very stable potassium cobalticyanide, $K_3Co(CN)_6$, analogous to the ferricyanide. A more perfect reaction occurs on treating the cold solution with chlorine or bromine. The resultant solution of potassium cobalticyanide is not decomposed by boiling

with mercuric oxide. This reaction enables nickel to be separated from cobalt, the former of which is wholly precipitated, and remains as NiO on igniting the precipitate. The same reaction distinguishes cobalticyanides from ferrocyanides and ferricyanides. Cobalticyanides give no precipitate with ferric salts. They are completely precipitated from acid solutions by nickel sulphate, the precipitate leaving $Ni_3 + Co_3$ by ignition in the air and subsequent reduction in hydrogen. Mercurous nitrate completely precipitates cobalticyanides from neutral solutions, the precipitate leaving Co_3O_4 on ignition. Cobalticyanides are completely decomposed by heating with concentrated sulphuric acid. By treatment with fuming nitric acid, potassium cobalticyanide yields a red substance stated to have the composition, $KH_2Co_3(CN)_{11} + H_2O$ (Jackson and Comey, *Ber.*, 1896, **29,** 1020).

Platinocyanides.

The platinocyanides are of interest from their remarkable fluorescent properties, which cause them to appear strongly dichroic. They become visible when subjected to the Roentgen dark rays, and a surface painted with barium platinocyanide and exposed while still moist to these rays, glows wherever it has not been protected by a metallic or other screen impervious to the rays. In this manner the effects of Roentgen rays may be rendered visible without resource to photography.

The platinocyanides of the light metals are soluble in water and crystallize well. Those of the heavy metals are mostly insoluble and can be prepared by precipitation. The platinocyanides are extremely stable, even boiling sulphuric acid decomposing them but slowly. When treated in solution with nitric acid, chlorine or bromine, the platinocyanides form addition compounds. Thus on passing chlorine into a hot solution of potassium platinocyanide, the compound $K_2Pt(CN)_4Cl_2 + 2H_2O$, is deposited in colourless crystals on evaporation. On treating these with a stong solution of the platinocyanide, they are converted into copper-red needles which are said to have the composition: $5K_2Pt(CN)_4, K_2Pt(CN)_4Cl_2 + 18H_2O$. This compound when boiled with sodium hydroxide, yields potassium platinocyanide, chloride and ferricyanides. The platinocyanides are not decomposed by digestion with mercuric oxides. Mercuric chloride throws down white mercuric platinocyanide, $HgPt(CN)_4$. Mercurous nitrate in

small quantity yields a white precipitate, but when added in excess, a highly characteristic bright blue precipitate is obtained. Cupric sulphate yields a flocculent blue or green precipitate containing $CuPt(CN)_4$, which when suspended in water and decomposed by hydrogen sulphide, yields free hydroplatinocyanic acid. On evaporating the filtrate to dryness, and recrystallising the residue from a mixture of alcohol and ether, the acid is obtained in bluish-black hydrated prisms, or greenish-yellow needles, having a coppery or golden lustre, which turn yellow and deliquesce on exposure to air.

Potassium platinocyanide, $K_2Pt(CN)_4 + 3H_2O$, is obtained by dissolving ammonium chloroplatinate and a little potassium hydroxide in a concentrated boiling solution of potassium cyanide, and recrystallising the product from water. Schertel (*Ber.*, 1896, **29,** 204) prepares the salt by dissolving well washed and recently-precipitated platinum sulphide in a solution of potassium cyanide, and concentrating the resultant colourless liquid. If commercial potassium cyanide containing much sodium cyanide be employed, crystals of the composition $KNaPt(CN)_4 + 3H_2O$ are obtained, and from the mother liquor sodium platinocyanide, $Na_2Pt(CN)_4 + 3H_2O$, crystallises out in long, transparent, colourless needles, permanent in the air, and not fluorescent. The salt may also be conveniently prepared by precipitating a solution of potassium platinocyanide with copper sulphate, and treating the washed precipitate with a slight excess of sodium hydroxide.

Potassium platinocyanide forms yellow rhombic crystals exhibiting a blue dichroism. On exposure to air it effloresces and becomes nearly white. The salt is readily soluble in hot water, but is in great part deposited on cooling. Cold concentrated sulphuric acid decomposes it with formation of platinous cyanide.

Barium platinocyanide, $BaPt(CN)_4 + 4H_2O$, may be prepared in a manner similar to the potassium salt; or by passing hydrocyanic acid vapours into a solution of platinic chloride holding barium carbonate in suspension, until oxygen and carbon dioxide cease to be evolved:

$$PtCl_4 + 3BaCO_3 + 4HCN = BaPt(CN)_4 + 2BaCl_2 + 2H_2O + 3CO_2 + O.$$

Barium platinocyanide forms monoclinic prisms which appear green in the direction of the principal axis, but sulphur-yellow with a blue-violet sheen in the direction at right angles to the axis. The salt dissolves in about 33 parts of cold water, but is considerably more soluble at the boiling point.

Magnesium platinocyanide, $MgPt(CN)_4 + 7H_2O$, is obtained by

precipitating the barium salt with magnesium sulphate. It crystallises in large prisms, which are powerfully fluorescent and dichoric. When crystallised from alcohol and from water at 70°, a yellow salt containing $6H_2O$ is obtained, which at 100° is converted into a white hydrate containing $2H_2O$, and this at 180° gives a yellow anhydrous salt.

Cyanates.

A series of compounds having the composition of cyanates or oxycyanides are produced by the action of oxidising agents on cyanides. The metallic cyanates are obtainable by the following reactions:

(1) By passing cyanogen gas into the solution of the hydroxide of an alkali or alkali earth metal:

$$2 KOH + (CN)_2 = KCNO + KCN.$$

(2) By heating a carbonate of the alkali metal to low redness with mercuric cyanide.

(3) By the electrolysis of a solution of the corresponding cyanide, the cyanate being formed at the anode.

(4) By fusing a cyanide or ferrocyanide with an oxidising agent, such as manganese dioxide, red lead, litharge, potassium dichromate, nitre, etc.

(5) By the action of a highly alkaline solution of a hypochlorite on urea.

(6) By the action of a hypobromite on a cyanide.

(7) By the action of an alkaline solution of permanganate on a cyanide.

The alkyl cyanates are compounds of great theoretical interest, but they have received no practical application.

Cyanic Acid or Hydrogen Cyanate.—This compound cannot be prepared by the action of mineral acids on metallic cyanates, since, at the moment of its formation, the greater part is decomposed into carbon dioxide and ammonia, the latter compound remaining in combination with the mineral acid used:

$$KCNO + H_2O + 2HCl = KCl + NH_3, HCl + CO_2.$$
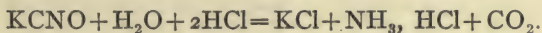
Sufficient cyanic acid escapes decomposition to give to the evolved gas a pungent odour which excites tears.

Cyanic acid may, however, be obtained by heating its polymer, anhydrous cyanuric acid, nearly to redness in a current of carbon dioxide. A mixture of urea with phosphoric anhydride, or of uric acid with sulphuric acid and manganese dioxide, may be substituted for the cyanuric acid.

Cyanic acid is a colourless liquid, having an extremely pungent odour resembling that of sulphurous or glacial acetic acid. It is extremely unstable, becoming rapidly polymerised into a snow-white insoluble substance called cyamelide, $(CHNO)_n$.

The sp. gr. of cyanic acid at $0°$ is 1.140, and the vapour tension 1.50. The aqueous solution is only stable below $0°$, decomposing at higher temperatures into carbon dioxide and ammonia.

$$HCNO + H_2O = CO_2 + NH_3.$$
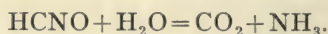
Cyanic acid is not soluble in alcohol, but reacts with it forming allophanic acid esters.

Two isomeric forms of cyanic acid have a possible existence, represented respectively by the following formulæ:

Normal cyanic acid Iso-cyanic acid
(Cyanogen hydroxide) (Carbylimide)
$\left.\begin{matrix} CN \\ H \end{matrix}\right\}$ O; or HO.C≡N $\left.\begin{matrix} CO \\ H \end{matrix}\right\}$ N; or H.N=CO
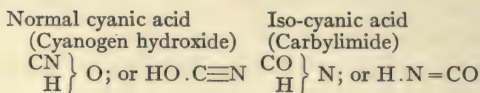
Only one modification of cyanic acid is known in the free state, and the constitution of this is not certain, some authorities regarding it as the normal acid and others as isocyanic acid. The same difference of opinion exists as to the constitution of the ordinary metallic cyanates (see below). The alkyl normal cyanates are almost unknown, as they polymerise with great facility into the corresponding cyanurates. The alkyl isocyanates are obtainable as volatile pungent liquids, readily polymerising to isocyanurates.

Metallic Cyanates.—As stated above the molecular constitution of the metallic cyanates is uncertain. Some authorities, arguing from the fact that potassium cyanate, when distilled with potassium ethyl sulphate, yields ethyl isocyanate, regard the ordinary potassium salt as an isocyanate. Other chemists think too much stress is laid on this and allied facts, since frequent observations have shown that normal cyanic compounds readily isomerise. Similarly, alkyl thiocyanate readily changes into the isothiocyanate, and the normal cyanuric esters change to the corresponding isocyanuric esters. It is conceiv-

able that the mother substances may possess two constitutional form-
ulæ, *i.e.*, that they are isodynamic, and by the wandering of an atom of
hydrogen their atoms may sometimes arrange themselves in one, and
sometimes in the other form, and that they may accordingly show the
reaction of either.

Potassium Cyanate, KCNO, is the most important salt of cyanic
acid. It is formed by passing cyanogen or cyanogen chloride in
potassium hydroxide solution, by glowing potassium carbonate in a
cyanogen atmosphere and by melting potassium ferrocyanide with
potassium carbonate and potassium cyanide.

Volhard (*Ann. Chem. Phys.*, **259,** 377) obtained potassium
cyanate by the oxidation of a cold solution of potassium cyanide with
permanganate in the presence of potassium hydroxide. Tarugi
(*Gaz. chim. ital.*, 1902, [*ii*], **32,** 383) uses 1 molecule of potassium
cyanide with 1 molecule of potassium persulphate dissolved in water.
He lets the mixture stand one day in the cold with an excess of ammonia,
when it is heated for about a quarter of an hour on the water-bath and
claims to obtain quantitative results. The best practical method of
preparing potassium cyanate consists in fusing potassium cyanide
with litharge or manganese dioxide, or according to Erdmann (*Ber.*,
1893, **26,** 2438) potassium ferrocyanide with potassium dichromate.

Erdmann proceeds as follows: Four parts of well dried potassium
ferrocyanide in powder is mixed with three parts of dried pulverised
potassium dichromate. The mixture is gradually introduced, in small
quantities at a time, into a large iron crucible, which is heated to a
point just short of red heat. Oxidation takes place, and is indicated
by a glowing of the mass introduced. As soon as one portion has
ceased to glow, the next is introduced, and so on, the contents of the
iron crucible being stirred with an iron spatula. The temperature
must not be allowed to rise high enough to effect the melting of the
resultant mass, which should remain porous and spongy. After
cooling, the product is powdered and extracted with three times its
weight of boiling methylated spirit of 80% strength. The solution is
filtered, and on cooling deposits potassium cyanate as a perfectly
white crystalline powder. The mother-liquor is used to extract the
product a second time, and this proceeding is repeated until no further
crystals are deposited on cooling. The crystalline powder is then
washed several times with small quantities of ether.

Potassium cyanate crystallises in colourless scales, with a sp. gr. of

2.048, fusible below a red heat to a colourless liquid. It is readily soluble in water, and tolerably soluble in boiling rectified spirit, but is insoluble in absolute alcohol. Potassium cyanate is not decomposed by exposure to or even by ignition in dry air, but in presence of moisture or by evaporation of its aqueous solution it suffers hydrolysis according to the following equation:

$$2KOCN + 3H_2O = K_2CO_3 + 2NH_3 + CO_2.$$
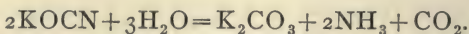
This reaction is the cause of the ammoniacal smell of deliquesced commercial potassium cyanide, which often contains much cyanate. On addition of moderately concentrated sulphuric or hydrochloric acid to potassium cyanate, the greater part of the cyanic acid liberated is decomposed into ammonia and carbon dioxide. Traces of the acid escape this change, and hence the carbon dioxide evolved has an extremely pungent odour resembling that of sulphurous acid, and most powerfully affects the eyes. The odour is slowly but very well developed by treating a solution of the cyanate with potassium hydrogen tartrate. Acetic acid, and some other acids, when added to a solution of potassium cyanate, throw down a crystalline precipitate of potassium hydrogen cyanurate, $KH_2(CNO)_3$. On adding ether and strong hydrochloric acid to a solution of potassium cyanate, some of the liberated cyanic acid is polymerised to cyanuric acid, which dissolves in the ether.

Ammonium Cyanate, NH_4CNO, is obtained by mixing cyanic acid vapours with ammonia in excess, when it is deposited in minute crystals which effervesce with acids. The salt may also be obtained by decomposing silver cyanate by a solution of ammonium chloride; or barium, lead, or potassium cyanate by ammonium sulphate. Ammonium cyanate is extremely unstable, being converted by boiling or evaporating the solution into urea, probably with previous conversion into the isocyanate:

$$NH_4.O.CN = NH_4.N.CO = NH_2.CO.NH_2.$$
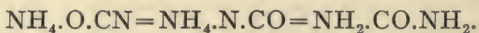
Barium cyanate, $Ba(CNO)_2$, separates in crystals when mixing alcoholic solutions of barium acetate with potassium cyanate.

Calcium cyanate, $Ca(CNO)_2$, was recently manufactured on a large scale. Owing to its high content of nitrogen, and ready decomposition with formation of ammonia, it was intended to employ calcium

cyanate as a fertiliser. It is prepared as follows: A mixture of lime-stone and coke is submitted to a preliminary temperature of 1,500° in an electric blast furnace, and is then superheated in the same furnace to 2,500° in presence of a large excess of pure nitrogen, and then finally oxidised by means of air, the oxygen of which is retained by the product while the nitrogen conveys the heat due to the oxidation into the electric chamber. The operation must be conducted in a large furnace, so that the calorific yield may be sufficiently economical.

Cobalt potassium cyanate, $K_2Co(CNO)_4$, is precipitated in dark blue quadratic crystals on adding a solution of cobalt acetate (or cobalt nitrate with potassium acetate) to a solution of potassium cyanate. The reaction has been applied to the detection of cyanate in commercial potassium cyanide.

Lead cyanate, $Pb(CNO)_2$, is a crystalline salt, nearly insoluble in hot water.

Silver cyanate, $AgCNO$, forms a crystalline precipitate, somewhat soluble in boiling water, but 100 parts of water at 16° dissolve only 0.006 parts of the salt. Dilute nitric acid decomposes it with evolution of carbon dioxide and formation of silver and ammonium nitrates (see page 509).

Detection of Cyanates.

As stated above sulphuric acid both concentrated and dilute will decompose cyanates, forming ammonia and evolving carbon dioxide.

Silver nitrate forms a white cheese-like precipitate which is soluble in ammonia and nitric acid, thus differing from silver cyanide.

Cobalt acetate solution is coloured deep blue by the addition of cyanate solutions. If the solutions are diluted the colour disappears. Nearly all commercial cyanide contains cyanate, but the hydrocyanic acid must be driven off before the presence of cyanates can be detected. According to Schneider (*Ber.*, 1895, **28**, 1540) the test should be made by dissolving about 3 to 5 grm. of the commercial cyanide in about 30 to 50 c.c. cold water, passing carbon dioxide through for 1.5 hours; this drives off the hydrocyanic acid. 1 c.c. of the liquid is treated with 25 c.c. absolute alcohol, which precipitates the formed carbonate, and filtered. A few drops of acetic acid are added to the filtrate and then a few drops of an alcoholic solution of cobalt acetate. If 0.5% cyanate is present in the commercial cyanide, it can be plainly detected.

Estimation of Cyanates.

For the estimation of cyanates in commercial cyanide, see under estimation of the impurities in commercial cyanide, page 485.

Several methods of estimation have been based on Wöhler's reaction (*Gilbert's Ann.*, **43**, 157), whereby cyanates are converted by dilute nitric acid into nitrate, ammonia and carbon dioxide:

$$AgCNO + 2HNO_3 + H_2O = AgNO_3 + NH_4NO_3 + CO_2.$$
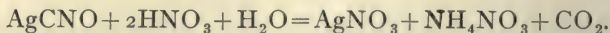
Allen (Commercial Org. Anal., 1896, **3**, 484) first published a method for the determination of cyanate, when existing in the form of pure potassium cyanate: 1 grm. of the sample is dissolved in very cold water, and precipitated without delay by an excess of a neutral solution of silver nitrate. The precipitate of silver cyanate is filtered off and washed slightly. It is then dissolved in a moderate excess of warm normal nitric acid, when the above reaction takes place. Hence, one mol. of cyanate will neutralise two of nitric acid. The liquid is filtered from any trace of insoluble matter and titrated with normal alkali. Each c.c. of normal acid neutralised by the cyanate represents 0.04055 grm. of KCNO in the sample taken. The same method can be applied for the estimation of cyanate in commercial cyanide, the carbonate being first separated by precipitating the cold solution with calcium nitrate. As the cyanide and any chloride which may be present will be precipitated as silver salts together with the cyanate, the subsequent treatment with warm dilute nitric acid will leave an insoluble residue, which must be separated before titrating with alkali.

Mellor (*Zeit. f. anal. Chem.*, 1901, **40**, 17) dissolves 20 grm. potassium cyanide, containing cyanate, in 100 c.c. water, treats with a calcium nitrate solution (free from chlorides) to precipitate any carbonate present. He then filters, washes with water and makes the filtrate up to 200 c.c. In 10 c.c. of the filtrate the cyanide contents are determined by titration with $N/10$ silver nitrate. To another 10 c.c. of the filtrate silver nitrate solution is added till no more precipitate appears, when all the cyanogen is present as silver cyanide and cyanate. The mixture is filtered off, washed with ice cold water and then washed into a beaker. Now 5 c.c. of N nitric acid is added, and the cyanide decomposed by heating to 50°, filtered and washed. The excess nitric acid is titrated with N sodium hydroxide. 1 c.c. of the N nitric used = 0.0405 grm. potassium cyanate. This method is not absolutely

accurate, and is liable to give too high results, because silver cyanide is very slightly soluble in dilute nitric acid.

Victor (*Zeit. f. anal. Chem.*, 1901, **40,** 462) recommends a much simpler method based on the same reaction. The solution is prepared in the same way, only he uses barium nitrate to precipitate the carbonates. The filtrate is made as before up to 200 c.c., two portions of 10 c.c. each are taken and treated with an excess of $N/10$ silver nitrate solution in 100 c.c. volumetric flasks. One of the flasks is filled to the mark, filtered through a dry filter and the excess of silver is estimated in an aliquot part of the same according to Volhard with $N/10$ thiocyanate solution (see page 552). In the second flask 10 c.c. dilute nitric acid are added before filling to the mark. It is then filtered through a dry filter and again the excess of silver is estimated according to Volhard. The difference of the two titrations gives the amount of silver which was combined with the cyanate. 1 c.c. of $N/10$ silver nitrate solution corresponds to 0.00806 grm. potassium cyanate.

Herting (*Zeit. f. anal. Chem.*, 1901, **40,** 585) doubts the correctness of the above methods and prefers to decompose the cyanate into ammonia and carbon dioxide with acid. 0.2 to 0.5 grm. of the potassium cyanide under investigation are dissolved in a little water, treated with dilute hydrochloric or sulphuric acid in a porcelain dish, and evaporated to dryness on the water-bath. The residue is taken up with water and the ammonia distilled over in the usual manner with milk of lime. The ammonia is taken up in $N/10$ sulphuric acid and the excess acid titrated with $N/10$ sodium hydroxide.

Evan (*J. Soc. Chem. Ind.*, 1909, **28,** 244) uses a similar method, but estimates the carbon dioxide. He dissolves about 1 grm. potassium cyanide in 100 c.c. water, acidifies with sulphuric acid and distils. The carbon dioxide is taken up in sodium hydroxide solution and precipitated as barium carbonate and weighed.

Polymers of Cyanic Acid.

Besides cyamelide, $(CNHO)_n$, which may possibly be identical with isocyanuric acid, various other polymers of cyanic acid exist, and are known either in the free state or as salts. Thus:

Fulminic acid, $H_2C_2N_2O_2$, is unknown in the free state, but the metallic fulminates are explosive salts typified by fulminating mercury, used in the preparation of percussion caps. The constitutional formula of fulminic acid is not known with certainty. The following are

the formulæ suggested by Divers and Armstrong (*Jour. Chem. Soc.*, 1885, **47**, 79):

$$\text{Divers} \qquad\qquad \text{Armstrong}$$

$$O\begin{cases} N:C.OH \\ \ \ \ | \\ N:CH \end{cases} \qquad N\begin{cases} C.OH \\ \ | \\ C(N.OH) \end{cases} or \begin{cases} N.CH \\ |\ | \\ O\ C(N.OH) \end{cases}$$
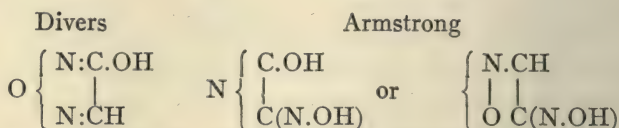
The question has recently been reviewed by Scholl (*Ber.*, 1890, **23**, 3505) who considers, with Steiner, that the properties and modes of formation of the fulminates are best explained on the assumption that fulminic acid has constitution of dioximinoethylene:

$$OH.N:C:C:N.OH.$$
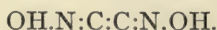
Mercuric fulminate, $HgC_2N_2O_2$, is obtained by warming alcohol with mercuric nitrate and nitric acid. It forms small silky crystals which explode with great violence when heated or struck. Silver fulminate is still more explosive. Concentrated hydrochloric acid decomposes the fulminates with formation of hydroxylamine hydrochloride and evolution of carbon dioxide.

Fulminuric acid, $HC_3H_2N_3O_3$, results from the action of a chloride or iodide of an alkali metal on mercuric fulminate.

Cyanuric acid, $(HCNO)_3$, is formed by the dry distillation of uric acid, by the action of heat on urea, and in various other reactions. Cyanuric acid is a compound of great theoretical interest, as also are the alkyl salts of its isomer isocyanuric acid, $(CO)_3(NH)_3$.

Thiocyanates, Sulphocyanides.

These salts are the sulphur analogues of the cyanates or oxycyanides. These salts are also called sulphocyanates, but such a term would be more appropriately applied to compounds of the formula $M(SO_2)CN$.

Like cyanic acid, thiocyanic acid exists in two isomeric forms, which are known in their metallic and alkyl salts, though only one modification (probably the normal) of the free acid has been isolated. The ordinary thiocyanic acid has the constitution $NC—SH$, and the isothiocyanic acid has the constitution $S=C=NH$. The normal salts of the alkyl radicals are known, but they change with great facility into the isomeric forms, which are preferably called thiocarbimides. The type of these compounds is allyl thiocarbimide, the volatile oil of mustard.

A polymeride of thiocyanic acid is known $(HCNS)_2$ and the methyl

ester of trithiocyanic acid (thiocyanuric acid), $(HCNS)_3$, has been obtained.

Notable quantities of thiocyanates occur naturally in the saliva and contents of the stomach, and they may also be detected in the urine. Hence thiocyanates appear to pass through the system unaltered. In the saliva their presence is directly indicated by the red colouration produced on addition of ferric chloride. For the detection of thiocyanates in urine the liquid is precipitated with baryta-water, the filtrate evaporated to a syrup, extracted with alcohol, the solution so obtained again evaporated, the residue redissolved in water, the solution decolorised with animal charcoal, and tested by ferric chloride, etc.

Bruylants (*J. f. prakt. Chem.*, [*v*], **18**, 104) found 0.0748 grm. potassium thiocyanate in the saliva and 0.00292 grm. ammonium thiocyanate per litre in the urine. He found in ox blood 0.00075 grm., in ox liver 0.01 grm. and in cow's milk 0.0008 to 0.0024 grm. ammonium thiocyanate per litre. Kelling (*Zeit. f. Physiologische Chem.*, **18,** 397) observed the presence of thiocyanate salts in the intestines. According to Gscheidlen (*Jahresber.*, 1877, 1001) thiocyanates originate in the salivary gland.

Sinapine thiocyanates exist ready formed in the seeds of mustard and some allied plants, and the isothiocyanates of acrinyl and allyl are formed by the action of the ferment myrosin on the glucosides of white and black mustard respectively, while other cruciferous seeds yield identical or analogous compounds.

Thiocyanates are also present in notable quantities in the ammoniacal liquor and spent oxide obtained in the purification of coal gas, and in the liquors resulting from the lixiviation of the black ash produced in the Leblanc process of manufacturing sodium hydroxide. The recovery of sulphocyanides from these and allied sources and their manufacture on the large scale by various synthetical methods with a view to conversion into cyanides or ferrocyanides have formed the subject of various patents.

As a class the thiocyanates are very poisonous, and have as deleterious an effect on plants as on animals. In a case where a woman took ammonium sulphocyanide she was found unconscious, with rigidity of the muscles of the arms and jaws. In spite of attempts to revive her, convulsions ensued, followed by death in 16 hours from the first symptoms observed. A dose of 5 grains of ammonium thiocyanate is said to be immediately fatal.

Most of the metallic thiocyanates are soluble, the chief exception being the cuprous, mercurous, mercuric, lead, and silver salts. From a solution acidified with hydrochloric acid and filtered, thiocyanates precipitate copper salts only. The thiocyanates readily form double salts.

Thiocyanates form very easily by the direct addition of sulphur to cyanides. Thus by simply melting or heating with sulphur, or by treating aqueous solutions with alkali sulphides, salts of thiocyanate are formed.

Thiocyanic acid, HCNS, is prepared by decomposing mercuric thiocyanate with hydrogen sulphide. It forms a colourless liquid, which crystallises below freezing temperature and easily decomposes into hydrocyanic acid and perthiocyanic acid, $C_2N_2H_2S_3$. The aqueous solution does not decompose so easily and is far more stable than cyanic acid. It has an odour similar to acetic acid and is not poisonous. When boiled, part of the thiocyanic acid distils without decomposition and the rest decomposes into ammonia, carbon disulphide and carbon dioxide:

$$2HCNS + 2H_2O = CO_2 + CS_2 + 2NH_3.$$
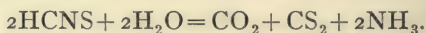
If thiocyanic acid is treated with hydrogen sulphide it forms carbon disulphide and ammonia:

$$HCNS + H_2S = CS_2 + NH_3.$$
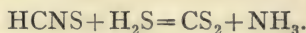
When treated with sulphuric acid it decomposes to ammonia and carbon oxysulphide.

Ammonium thiocyanate, ammonium sulphocyanide, NH_4CNS, is formed by the action of ammonium polysulphide on hydrocyanic acid, also by reaction of carbon disulphide on ammonia.

It is prepared according to Schulze (*J. f. prakt. Chem.*, **27,** 518) by mixing 600 grm. alcohol (95%) with 800 grm. ammonia (sp. gr. 0.912) and 350 to 400 grm. carbon bisulphide, heating the mixture several hours using reflux condenser.

Ammonium thiocyanate is a product of the distillation of coal and is found in considerable quantities in the ammonia liquor and spent oxide of the gas works.

Ammonium thiocyanate crystallises in colourless plates or prisms, is deliquescent and has a sp. gr. of 1.3075. It melts at 159°. When kept nearly at this temperature for some time, it suffers conversion into thio-urea:

$$NH_4.S.CN = NH_2.CS.NH_2.$$
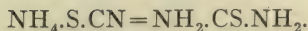
At 180° to 190° it evolves hydrogen sulphide, carbon disulphide, and ammonia, and leaves a residue containing guanidine thiocyanate. At a still higher temperature melam, $C_6H_3N_{11}$, is obtained.

100 parts of water dissolve 122 parts of ammonium thiocyanate at 0°, and 162 parts at 20°. In dissolving this salt in water a considerable amount of heat is absorbed, and according to Phipson (*Chem. News*, 1869, 19, 109) if 500 grm. ammonium thiocyanate are dissolved in 500 grm. of water at 96° the temperature falls to $-2°$. This salt is also easily soluble in alcohol.

Commercial ammonium sulphate often contains thiocyanate, which prejudicially affects its application as a fertiliser. Jumeau (*Analyst*, 1893, **18,** 135) has described a sample which contained 9% ammonium thiocyanate and a similar proportion of sodium sulphate, but such samples are very unusual. In estimating the amount of thiocyanate, it is desirable, though not essential, to extract the sample with methylated spirit, and evaporate an aliquot part of the solution, and estimate the thiocyanate in the residue by one of the methods given below.

Ammonium thiocyanate acts as a powerful poison to animals and also exerts a toxic action on plants. Maize is particularly sensitive to its action; as small a quantity of 9 kilogrm. of thiocyanate per hectare shows its influence in the generally deteriorated appearance of the plants. It was found that, when watered with a solution containing 0.01 grm. of ammonium thiocyanate per litre, old plants with 6 to 8 leaves were uninjured, but sickened at once with a solution of twice the strength, and 0.1 grm. per litre proved fatal almost immediately. Seeds lose their power of germination when steeped in 0.25% solution of ammonium thiocyanate.

Potassium thiocyanate, potassium sulphocyanide, KCNS, is prepared by melting together 17 parts potassium carbonate with 3 parts sulphur and adding to the mixture 46 parts of dehydrated potassium ferrocyanide. When all the potassium ferrocyanide has been decomposed, the mass is heated higher decomposing some of the potassium thiosulphate, and is then allowed to cool. The melt is extracted with water, filtered, neutralised with sulphuric acid and partly evaporated. The potassium sulphate is precipitated by the addition of alcohol and the filtrate is evaporated for crystallisation.

The salt crystallises in colourless, anhydrous, deliquescent needles having a sp. gr. of 1.886 to 1.906. 100 parts water dissolve 177 parts potassium thiocyanate at 0° and 217 parts at 20°, with considerable lowering of temperature. Rudorff (*Ber.*, 1861, **2,** 69) states that by dissolving 150 parts potassium thiocyanate in 100 parts of water at 10.8°, the temperature sinks to −23.7°. The aqueous solution gradually decomposes at ordinary temperatures and more rapidly when boiled, with evolution of ammonia. The solution dissolves silver chloride, cyanide and thiocyanate.

When strongly heated without contact of air, potassium thiocyanate evolves carbon disulphide and leaves a residue containing potassium sulphide and potassium mellonide, $K_3C_9N_{13}$, which crystallises with $3H_2O$ from hot water.

Nollner (*Jahresber.*, 1856, 443) claims that when melting potassium thiocyanate (also sodium thiocyanate) in a porcelain crucible it is coloured brown-green to indigo blue, returning to its original colour when cooled, and Giles (*Chem. News*, 1901, **83,** 61) found that the colouring was increased by the addition of sulphur but destroyed by adding potassium hydroxide or carbonate. An atmosphere of hydrogen also destroyed the colour, carbon bisulphide being evolved, leaving potassium cyanide behind.

If potassium thiocyanate is oxidised in acid solution with potassium permanganate it forms potassium cyanide and potassium sulphate. However, when oxidised in alkaline solution it forms potassium cyanate and potassium sulphate. Both nitric and nitrous acid, when added to a concentrated solution of potassium thiocyanate form a temporary blood-red colour.

According to Goppelsrœder (*Polytech. Jour.*, **254,** 83) perthiocyanogen is formed at the positive pole, as a yellow amorphous powder, electrolysing an aqueous solution of potassium thiocyanate. This same product is formed by oxidising the solution with potassium. persulphate according to Pawlewsky (*Ber.* 1900, 33, 3164).

Perthiocyanogen, $C_3HN_3S_3$, is an amorphous, deep yellow substance, insoluble in water, alcohol or ether, and unaffected by dilute alkalies or acids. It has been applied under the name of "canarin" for printing calico, the colour being formed in the fibre, and developed in a manner similar to aniline black. Perthiocyanogen acts as a mordant to many of the coal-tar dyes, such as methylene blue, aniline green, aniline red, etc. (Dingler's *Polyt. Jour.*, 1884, 251, 41).

Potassium isothiocyanate, potassium thiocarbimide, K.NCS, is the type of the metallic isothiocyanates. It is obtained by heating perthiocyanic acid with alcoholic potassium hydroxide. The crystals are soluble in water. Potassium isothiocyanate is partially transformed into the normal salt by repeatedly evaporating its solutions, and by fusion undergoes complete conversion. The reactions of the isothiocyanates differ from those of the normal salts in many respects. Thus, the silver salt is light yellow, and but slightly soluble in ammonia. With zinc chloride, thiocyanates give no reaction, but the isothiocyanates give a voluminous deep yellow precipitate, and with neutral ferric chloride a brown colouration, disappearing on addition of an excess of the iron salt. Cupric sulphate gives a greenish-yellow precipitate.

Barium thiocyanate, $Ba(CNS)_2$, is produced by boiling ammonium thiocyanate with baryta water, or by decomposing cuprous thiocyanate (from spent oxide or gas liquor) with barium sulphide. It forms long deliquescent needles, commonly stated to contain $2H_2O$ (but according to Tscherniac, $3H_2O$), and very soluble in water and alcohol. Its chief application is for the manufacture of aluminum thiocyanate.

Aluminium thiocyanate, $Al(CNS)_3$, is obtained by the double decomposition of aluminium sulphate with barium or calcium thiocyanate (*J. Soc. Chem. Ind.*, 1881, **1,** 64 and 364). Any red colour due to the presence of traces of iron can be removed by agitation with ether. Aluminium thiocyanate now receives extensive application in calico-printing.

The sulphocyanides of aluminium and other metals are used in dyeing and calico-printing for three distinct purposes: as a resist for aniline black; as an addition to the ordinary alizarin-red printing colour in order to resist the action of iron; and as the mordant for alizarin-red instead of acetates (Lauber and Storek, *Jour. Soc. Chem. Ind.*, 1882, **2,** 359). When applied to the last purpose thiocyanates are found to produce greater brilliance of tint and fastness on the fibre, apparently from the gradual manner in which they under go decomposition on steaming.

A soluble basic sulphocyanide of aluminium, containing $Al(OH)_2$ (CNS), is obtained by dissolving hydrated alumina in aluminium thiocyanate.

Cuprous thiocyanate, $Cu_2(CNS)_2$, is one of the most insoluble salts of the series. Cuprous sulphocyanide occurs in commerce under the

name of "white paste," which is obtained by acting on ammonium thiocyanate with a mixture of cupric and ferrous sulphates. A 47% paste is commonly sold. It should be tested for iron, barium sulphate, and soluble matters, and the thiocyanate estimated by boiling the paste with sodium hydroxide, acidulating the filtrate with dilute sulphuric acid, and titrating with permanganate.

Lead thiocyanate, $Pb(CNS)_2$, forms a yellowish-white crystalline precipitate, somewhat soluble in hot water and readily soluble in water acidified with hydrochloric acid.

Mercuric thiocyanate, $Hg(CNS)_2$, is obtained as a sparingly soluble, white, crystalline precipitate on adding a soluble thiocyanate to a strong solution of mercuric nitrate or chloride. It is soluble in excess of the precipitant and in dilute hydrochloric acid. On heating mercuric thiocyanate in a test-tube, or on kindling the powder, it ignites and swells up enormously, giving off sulphur dioxide, mercurial vapours, etc., and leaving a very porous bulky grey or brown mass containing mellon, $C_3H_3(NH)_3C_3H_3$.

"Pharaoh's serpents' eggs" consist of mercuric thiocyanate. The statement in some text-books that "Pharaoh's serpents' eggs" consist of mercurous thiocyanate is erroneous. Mercurous thiocyanate is extremely unstable, rapidly becoming grey from the separation of metallic mercury. On treatment with hot water "Pharaoh's serpents' eggs" yield a solution which gives a yellow precipitate with sodium hydroxide. On filtering off the precipitated mercuric oxide, the filtrate gives the reactions of a thiocyanate after being acidified with dilute nitric acid. Several cases of poisoning have occurred owing to children swallowing "Pharaoh's serpents' eggs."

Detection of Thiocyanates.

The following reactions of analytical and general interest are given by a solution of potassium or other soluble thiocyanate:

On addition of a mineral acid dilute solutions of thiocyanates suffer no immediate change, but strong solutions are decomposed with formation of thiocyanic acid and other products, and separation of perthiocyanic acid (Klason, *Jour. f. prakt. Chem.*, [*ii*], **36,** 57).

On distillation with dilute sulphuric acid, potassium thiocyanate yields thiocyanic acid, HCNS, as a liquid of a pungent odour. Part of the acid is decomposed in a manner analogous to cyanic acid:

$$2HCNS + 2H_2O = 2H_3N + CS_2 + CO_2.$$
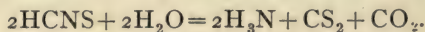
Another portion is split thus: $3HCNS = HCN + H_2C_2N_2S_3$. (Klason, *Jour. f. prakt. Chem.*, 1887, [*iii*], **35**, 789).

From a hot solution of potassium thiocyanate, nitric acid, chlorine, or a mixture of hydrochloric acid and potassium chlorate precipitate a yellow substance containing $C_3HN_3S_3$, inaptly called perthiocyanogen (see page 546).

Concentrated sulphuric acid reacts vehemently with thiocyanates and evolves acrid vapours consisting of formic acid, sulphur dioxide, carbon dioxide and carbon oxysulphide, sulphur separating out.

Silver nitrate precipitates from solutions of soluble thiocyanates white curdy silver thiocyanate, AgCNS, insoluble in dilute nitric acid, but soluble in ammonia and in soluble thiocyanates.

Cupric sulphate produces no immediate change in a weak solution of thiocyanates, but in a strong solution precipitates black cupric thiocyanate, $Cu(CNS)_2$, which turns white on standing. If sodium sulphite, sulphurous acid, or other reducing agent be added together with the cupric solution, a white precipitate, consisting of cuprous thiocyanate, $Cu_2(CNS)_2$, is immediately formed. The precipitate is insoluble in water and saline solutions, and nearly insoluble in dilute sulphuric and hydrochloric acids, but dissolves in ammonia.

Ferrous sulphate, if quite free from ferric salt, occasions no change in solutions of thiocyanates.

When added to a slightly acid solution of a soluble thiocyanate, ferric sulphate or chloride produces a deep red colouration, owing to the formation of soluble red ferric thiocyanate, $Fe(CNS)_3$. This is a most delicate and characteristic reaction for ferric salts and thiocyanates. The colour is not destroyed by boiling, or by cold dilute mineral acids (distinction from acetates and formates). The fixed alkalies and ammonia precipitate brown ferric hydroxide, and thus destroy the colour. The colour is instantly destroyed by mercuric chloride (distinction from meconates) or by excess of silver nitrate (distinction from formates and acetates). In presence of ferrocyanide, excess of ferric solution should be added and the liquid filtered from the precipitate of Prussian blue, when the red colour will become apparent. In presence of ferricyanide, the dark coloured solution should be largely diluted.

The thiocyanate reaction is not obtained in a solution of a ferric salt to which sodium acetate or oxy-organic compounds, as tartaric acid, etc., have been added, until the solution is strongly acidified with

hydrochloric acid. Nor does the test answer for solutions of basic ferric salts, obtained by digesting dilute ferric chloride with ferric hydroxide, or by adding ammonium carbonate to ferric chloride solution as long as the precipitate is redissolved. Very dilute ferric solutions free from acidity are inactive toward thiocyanate solutions at ordinary temperatures, and somewhat stronger solutions do not give the colour when hot. This is explained by the complete hydrolytic dissociation which occurs in dilute solutions at ordinary temperatures, and in stronger solutions at higher temperatures. Acidifying of the solution, however, always renders the thiocyanate test applicable.

When the red liquid produced by adding a ferric salt to a solution of a thiocyanate is shaken with ether, the colour passes wholly into the ether. By evaporation of the aqueous or ethereal solution of ferric thiocyanate, intensely deep red crystals are obtained, readily soluble in water, alcohol and ether.

According to Kruss and Moraht (*Ber.*, 1889, **22**, 2061) the red colouration produced by adding potassium thiocyanate to a ferric salt is due to the formation of a compound containing $Fe(CNS)_3, 9KCNS + 4H_2O$. This salt may be obtained by adding the calculated quantity of potassium thiocyanate to a neutral solution of ferric thiocyanate. It is insoluble in dry ether, but is decomposed by moist ether into potassium thiocyanate, which is insoluble, and ferric thiocyanate, which dissolves in the ether with red colour. A compound containing $Fe(CNS)_3, 3KCNS$ has also been obtained.

A solution of molybdic acid or molybdate of ammonium in hydrochloric acid gives a red colour with a thiocyanate which, like the similar colour produced by ferric salts, is removed from its aqueous solution by agitation with ether.

When a thiocyanate is treated with zinc and hydrochloric acid, hydrogen sulphide is evolved (which will blacken lead paper held over the tube), and methylamine, $(CH_3)NH_2$, is formed in the solution.

When added to a solution of a thiocyanate acidified by dilute hydrochloric acid, a solution of potassium permanganate is instantly decolourised with formation of hydrocyanic acid and sulphuric acid.

Estimation of Thiocyanates.

For the gravimetric estimation of thiocyanate compounds the method suggested by Alt (*Ber.*, 1899, **22**, 3258) is used.

The compound is oxidised with nitric acid and the formed sulphuric acid is precipitated as barium sulphate:

$$HCNS + 3O + H_2O = HCN + H_2SO_4.$$
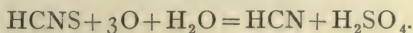
About 1 grm. of the thiocyanate salt is dissolved in a little water to which is added an excess of the calculated amount of barium chloride crystals. It is then strongly acidified with nitric acid and heated to boiling, until all the hydrocyanic acid has been driven off. It is now diluted with hot water and filtered, and the barium sulphate is estimated in the usual manner. 233.44 parts of barium sulphate precipitated correspond to 48.08 parts of thiocyanate ($-CNS$).

The above method can be used for the estimation of ammonium thiocyanate in the spent gas purifying mass, provided sulphur compounds, except sulphates, which can be oxidised to sulphates with nitric acid, are not present. 50 grm. of the spent purifying mass are allowed to stand for 12 hours with 500 c.c. water in a 1,000 c.c. volumetric flask, the flask is then filled to the mark and 30 c.c. additional water added to compensate the 50 grm. substance used. It is now shaken and filtered. 50 c.c. of the solution (2.5 grm.) are treated with an excess of barium chloride, heated, any sulphate present being precipitated as barium sulphate, which is filtered off and washed. The filtrate is strongly acidified with nitric acid and proceeded with as described above.

If thiosulphate or sulphite compounds are present in the spent purifying mass, then instead of treating 50 c.c. of the solution with barium chloride, a dilute solution of copper sulphate is added (about 1 grm. $CuSO_4$) and sulphur dioxide is passed into the solution for a short time, after which it is allowed to stand so that all the cuprous thiocyanate precipitated settles. The dried precipitate, together with the filter paper is mixed with sulphur and ignited in a current of hydrogen, until constant weight has been reached. 1 molecule cuprous sulphide corresponds to 2 molecules ammonium thiocyanate.

In the absence of other reducing agents, thiocyanates may be directly titrated in the cold with dilute sulphuric acid and standard permanganate. The reaction is as follows:

$$5KCNS + 6KMnO_4 + 12H_2SO_4 = 11KHSO_4 + 5HCN + 4H_2O$$

or more simply

$$HCNS + O_3 = HCN + SO_3.$$
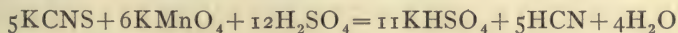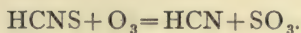
Each c.c. of $N/10$ permanganate decolourised represents 0.000967 grm. –CNS. The solution should, however, be standardised by pure potassium or ammonium thiocyanate.

According to Klason (*J. f. prakt. Chem.*, **36**, 74), the results obtained by titration with permanganate are always too low, the error being greater as the solution is more dilute. When the concentration is not less than decinormal, the result is about 1.5% below the truth, and a corresponding correction can be made.

Thiocyanates may be estimated by the above method in presence of simple cyanides and chlorides. For the estimation of the simple cyanide in such a mixture, the solution should be treated with excess of silver nitrate and the washed precipitate treated by Kjeldahl's process, metallic mercury being added and fuming sulphuric acid substituted for acid of 1.84 sp. gr. The ammonia formed is derived from the cyanide and thiocyanate, and the latter having been previously estimated by titration with permanganate, the cyanide can be deduced. The chloride can be estimated by difference, or directly estimated by oxidising the solution with permanganate in a solution acidified with sulphuric acid, boiling off the hydrocyanic acid, preferably in presence of granulated zinc, and then precipitating the chloride with silver nitrate.

For the estimation of cyanides, thiocyanates, ferrocyanides and ferricyanides when the four classes of salts occur together in a solution without heavy metals, the following method may be used: A measured quantity of the liquid is strongly acidified with hydrochloric acid, and precipitated with excess of ferric chloride. The precipitate of Prussian blue is filtered off, washed, and boiled with sodium hydroxide and the alkaline ferrocyanide produced filtered from the precipitated ferric hydroxide and estimated by titration with standard permanganate, or by one of the other methods described on page 510 *et seq.* The filtrate from the ferric chloride precipitate is treated with ferrous sulphate and the liquid again filtered. The precipitate consists of ferrous ferricyanide, which on boiling with sodium hydroxide yields soluble ferrocyanide as described. The filtrate from the precipitate produced by ferrous sulphate, which must contain excess of both ferrous and ferric salts, is treated with excess of soda and heated. It is then again acidified with hydrochloric acid, when a precipitate of Prussian blue will result, the amount of which represents the simple cyanide originally present. It may be boiled with alkali, and the ferrocyanide produced titrated as before, and calculated to CN. The thiocyanate

may be estimated in the filtrate from the various iron precipitates by precipitation as cuprous salt with cupric sulphate, or in a separate portion of the original liquid by oxidising with nitric acid and precipitating the resultant sulphate by barium. chloride.

Volhard's process for the estimation of silver is equally applicable to the titration of thiocyanates. The solution of the thiocyanate is acidified with nitric acid (previously well boiled to free it from nitrous acid), and a solution of ferric sulphate added. This produces a deep red solution of ferric thiocyanate. $N/10$ silver solution is next added from a burette until the red colour is replaced by a light brown and the latter is at last destroyed. The end point is better observed by adding excess of silver solution and titrating back with standard thiocyanate until a light tint is permanent on agitation. Each c.c. of $N/10$ silver nitrate used represents 0.0058 grm. of –CNS. In presence of ferrocyanides, excess of iron solution must be added and the liquid filtered before titrating with silver nitrate. The same plan is applicable in presence of ferricyanides if ferrous sulphate be substituted for the ferric salt. Cyanides may also be removed by ferrous and ferric salts and alkali, with subsequent acidification by dilute nitric acid, followed by filtration. Sulphides may be separated by filtering the solution after addition of iron salts and alkali, but before adding nitric acid. In presence of chlorides, bromides, or iodides, the process is still available, if, after the termination of the reaction, the silver precipitated be filtered off and treated in the following manner: The silver compound is dried and mixed with a large excess of pure sodium carbonate. The mixture is added gradually to fused nitre contained in a porcelain crucible. When the action is complete, the cooled mass is dissolved in water. The filtered liquid is neutralised by dilute nitric acid, and titrated with silver nitrate, using neutral potassium chromate as the indicator. The silver solution used represents the chloride, bromide, and iodide present. Its volume deducted from the amount originally required represents the silver solution corresponding to the thiocyanate. The thiocyanate may also be deduced from the amount of the sulphate formed on fusion with nitre. This plan does not necessitate the previous removal of the cyanides, ferrocyanides, or ferricyanides.

Any sulphide, cyanide, ferrocyanide, or ferricyanide is separated by iron salts, as in above process, but the liquid is rendered slightly acid by hydrochloric acid instead of nitric acid. The solution is then

treated with sodium sulphide and a solution of cupric sulphate added. Phosphates and other inorganic salts forming insoluble copper compounds may be got rid of by digesting the precipitate with cold dilute hydrochloric acid. The white precipitate of cuprous thiocyanate is filtered off, washed, dried at 100° and weighed. 121.3 parts of Cu_2 $(CNS)_2$ represent 58 of thiocyanogen. The process is not affected by bromides or chlorides, but is not directly applicable in presence of iodides, which are precipitated as white cuprous iodide. If the solution be acidified with sulphuric acid instead of hydrochloric acid before precipitating the thiocyanate as a cuprous salt, any chloride or bromide can be estimated in the filtrate as a silver salt in the usual manner.

The precipitate of cuprous thiocyanate is sometimes so finely divided that a clear filtrate is extremely difficult to obtain. This is especially the case in the presence of thiosulphates and certain other salts, such as co-exist with thiocyanates in gas liquor. For the estimation of thiocyanates in such cases, Dyson (*J. Soc. Chem. Ind.*, 1883, **3,** 231), recommends that 50 c.c. of the sample should be evaporated to dryness and the residue heated to 100° for three or four hours. If this prolonged heating be omitted, the cuprous thiocyanate will be precipitated in such a finely divided state as to render filtration almost impossible. The residue is digested with strong alcohol, rinsed on to a filter and washed with alcohol. Thiosulphates, which otherwise exercise a solvent action on cuprous thiosulphate, are left undissolved. The alcoholic filtrate is evaporated to dryness, the residue taken up with water, and the insoluble organic matter filtered off. A solution of ammonium thiocyanate is thus obtained tolerably free from other ammoniacal salts and from organic matter. The solution is then treated with sulphurous acid and cupric sulphate, gently warmed (not boiled), and the precipitate of cuprous thiocyanate filtered off.

A volumetric modification of the copper method of estimating thiocyanates has been described by Barnes and Liddle (*J. Soc. Chem. Ind.*, 1883, **3,** 231), which consists in boiling the solution with sodium bisulphite, and titrating with a standard solution of copper sulphate, the termination of the reaction being indicated by the immediate production of a brownish colour on bringing a drop of the liquid in contact with a drop of solution of potassium ferrocyanide on a white plate. The results are liable to several disturbing influences, but the process is useful under favorable conditions.

Rupp and Schiedt (*Ber.*, 1902, **35**, 2191) found that their method for the estimation of potassium ferrocyanide can also be used for the estimation of soluble thiocyanates, according to the following equation:

$$KCNS + 8I + 4H_2O = H_2SO_4 + 6HI + ICN + KI.$$
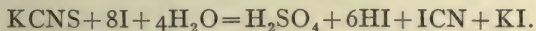
2 grm. of the soluble thiocyanate are dissolved in 1,000 c.c. water, 10 c.c. of the solution are taken and 25 c.c. $N/10$ iodine solution are run in from a burette, then 1 grm. solid acid sodium carbonate is added. The mixture is well shaken in a glass stoppered bottle and allowed to stand for about 1/2 hour, as the rate of reaction diminishes toward the end. The excess iodine is titrated with $N/10$ thiosulphate solution; no starch solution must be used in titrating the iodine, as cyanogen iodide also colours the starch blue, but the yellow colour of the iodine must act as its own indicator. Each c.c. of $N/10$ iodine solution used equals 0.0012156 grm. potassium thiocyanate. Thiel (*Ber.*, 1902, **35**, 2766) advises to acidify the solution before titrating the unused iodine with $N/10$ thiosulphate. The cyanogen iodide liberates free iodine and starch solution can be used to determine the end point of the titration. In this case each c.c. $N/10$ iodine used equals 0.001621 grm. potassium thiocyanate.

Feld (*J. f. Gasbel.*, 1903, **46**, 604) reduces the thiocyanate with nascent hydrogen, which is accomplished in hydrochloric acid solution by adding pieces of aluminium. The reaction is very vehement and the thiocyanic acid is quantitatively decomposed into hydrogen sulphide, ammonia and carbon:

$$3KCNS + 18HCl + 4Al = 3KCl + 2Al_2Cl_6 + 3NH_4Cl + 3C + 3H_2S.$$
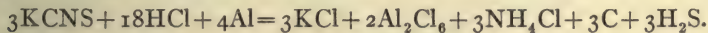
The hydrogen sulphide is driven off and absorbed in an excess of $N/10$ thiosulphate. 1 c.c. $N/10$ iodine solution corresponds to 0.004863 grm. potassium thiocyanate. This method is very complicated and requires considerable skill for its proper manipulation.

Very small quantities of thiocyanates, such as were met with in soda-lyes, may be estimated by acidifying the lye with hydrochloric acid, and adding zinc chloride. Any ferrocyanide of zinc is filtered off, and the filtrate coloured by ferric chloride. The tint is then compared colourimetrically with that produced by a known quantity of thiocyanate treated similarly. The results are only roughly approximate.

Insoluble thiocyanates may be fused with alkali carbonate and nitre, the sulphate produced being estimated by precipitation with

barium chloride. They may also be decomposed by hydrogen sulphide, the liquid filtered, ammoniacal solution of copper added, the sulphide of copper filtered off, and the filtrate treated with dilute sulphuric acid and sodium sulphite, when cuprous thiocyanate will be obtained (see page 553).

To determine the metals in thiocyanates, the salts may be decomposed by sulphuric acid and estimated in the usual manner.

Selenocyanides.

Selenium forms a series of salts, selenocyanides, precisely analogous to, but less stable than, the thiocyanates.

Potassium selenocyanide, KCNSe, is prepared by melting potassium cyanide with selenium in a current of hydrogen. This salt is soluble in water and is readily decomposable by acids, evolving hydrocyanic acid. Mixtures of selenium and tellurium can be separated by this method, as the tellurium does not readily form potassium tellurocyanide.

Silver selenocyanide, AgCNSe, is a white curdy precipitate closely resembling the thiocyanate. The cuprous salt is white and insoluble. On adding a drop of ferric chloride to a cold dilute solution of a selenocyanide, a red colouration is momentarily produced, probably owing to the formation of ferric selenocyanide, but the solution very rapidly becomes turbid and free selenium separates.

Cyanamide.

The direct combination of atmospheric nitrogen with earth alkali carbides has created an important and distinct interest in the salts of cyanamide, and recently several factories have been put in operation for the commercial manufacture of calcium cyanamide.

Cyanamide, H_2CN_2, (NH_2CN), is formed by causing cyanogen chloride to react with ammonia in ether. In the laboratory it is prepared by adding freshly precipitated mercuric oxide to an aqueous solution of thiourea:

$$CS(NH_2)_2 + HgO = H_2CN_2 + HgS + H_2O.$$
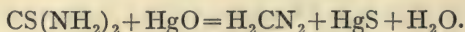
As soon as the solution shows no more sulphur reaction, it is filtered off, quickly evaporated and crystallised in vacuo over sulphuric acid. The residue is dissolved in ether to separate from dicyanamide and the solution evaporated.

Cyanamide forms colourless crystals, melting at 40°. It is very soluble in water, alcohol, and ether, but only slightly soluble in benzene, chloroform, and carbon bisulphide.

Sodium cyanamide, also disodium cyanamide, Na_2CN_2, is obtained according to Beilstein, Geuther (*Ann. Chem. Pharm.*, 1858, 108, 93) by passing carbon dioxide over sodium amide:

(1) $NaNH_2 + CO_2 = NH_2.CO.ONa$
(2) $NH_2.CO.ONa = NCO.Na + H_2O$
(3) $NCO.ONa + NaNH_2 = CN.NNa_2 + H_2O$.
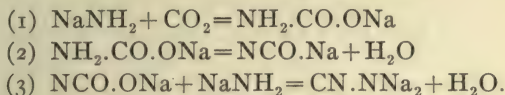
According to Pfleger, United States Patent, 1901, No. 671,709) sodium cyanamide is manufactured by melting sodium cyanide with sodium amide:

$$NH_2Na + NaCN = Na_2CN_2 + H_2$$
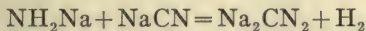
Of the alkali earth cyanamides, calcium cyanamide forms the most important commercial cyanamide salt. Frank and Caro (German Patent, 1895, No. 88,363) noticed that by passing nitrogen over barium carbide, the nitrogen was absorbed and formed barium cyanide. In carrying out similar experiments with calcium carbide, Rothe (*Zeit. f. angew. Chem.*, 1903, **16**, 535), Freudenberg (*Zeit. f. angew. Chem.*, 1903, **16**, 753), etc., found also that the nitrogen was absorbed, but instead of forming calcium cyanide, as was hoped, the product calcium cyanamide, $CaCN_2$, was obtained. This product was first used as a fertilizer by Freudenberg, and is known under the name of "Lime-nitrogen." As a commercial fertilizer lime-nitrogen is a mixture containing from 20 to 57% calcium cyanamide. It is very frequently mixed with gypsum, phosphates or other materials used as fertilizers. The literature is very extensive on this product and a complete bibliography is published by Jurisch (Salpeter und sein Ersatz, 1908), also in *Bulletin* No. 63 of the United States Bureau of Soils.

One of the most important properties of calcium cyanamide is that it is completely decomposed with superheated steam (Frank, German Patent, 1900, No. 134,289).

$$CaCN_2 + 3H_2O = CaCO_3 + 2NH_3.$$
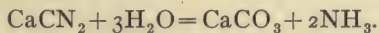
Cyanamide salts, according to Foersterling and Philipp (United States Patent, 1904, No. 796,713) are converted by a mineral acid into urea.

The alkali and earth alkali cyanamides are soluble in water. The author uses the following method for the estimation of cyanamide salts: A dilute solution of the cyanamide salt is prepared. In the case of alkali earth cyanamides, about 1 grm. substance is weighed off, placed in a 200 c.c. volumetric flask, together with about 100 c.c. water. The flask is placed on a water-bath and frequently agitated; the temperature should not be allowed to exceed 50°; after about 1/2 hour the flask is allowed to cool, filled to the mark, and 1 c.c. water added in excess to compensate for the insoluble residue. After shaking the flask, the contents are filtered through a dry filter and 50 c.c. of the filtrate taken. The solution is made ammoniacal and decomposed with ammoniacal silver nitrate, till no further precipitate is formed. The ammoniacal silver nitrate is prepared by dissolving 30 grm. silver nitrate in 375 c.c. water and adding 25 to 30 c.c. ammonia (27%) to the solution. The yellow precipitate of silver cyanamide is allowed to settle, which is greatly accelerated by thorough stirring, directly after the ammoniacal silver nitrate has been added. After standing about 2 hours the precipitate is filtered off and washed with cold water until no silver can be detected in the wash water. The silver cyanamide is dissolved in warm nitric acid and the silver titrated in the solution with N/10 ammonium thiocyanate, according to Volhard. 2 molecules ammonium thiocyanate correspond to 1 molecule cyanamide salt (see also page 552).

If the substance to be tested contains carbide, the acetylene gas absorbed must be driven out by passing air through the solution, silver acetylene being insoluble in ammonia. If this is not taken into consideration too high results are obtained.

To estimate the dicyanamide, 50 c.c. of the solution, as prepared for the cyanamide estimation, are taken and treated according to Brioux (*Ann. Chim. analyt.*, 1910, **15,** 341), with 20 c.c. silver nitrate (5%) and 20 c.c. potassium hydroxide (10%). The precipitate is filtered off, washed till practically free of potassium hydroxide and the nitrogen is then estimated by Kjeldahl's process. The difference of nitrogen found by this process and the titration process represents the dicyanamide present.

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